THE SIMPLE CARBOHYDRATES AND THE GLUCOSIDES

BY

E. FRANKLAND ARMSTRONG, D.Sc., PH.D.

P. P. Laidlaw

MONOGRAPHS ON BIOCHEMISTRY

EDITED BY

R. H. ADERS PLIMMER, D.Sc.

AND

F. G. HOPKINS, M.A., M.B., D.Sc., F.R.S.

MONOGRAPHS ON BIOCHEMISTRY

EDITED BY

R. H. ADERS PLIMMER, D.Sc.

AND

F. G. HOPKINS, M.A., M.B., D.Sc., F.R.S.

ROYAL 8VO.

THE DEVELOPMENT AND PRESENT POSITION OF BIOLOGICAL CHEMISTRY. By F. GOWLAND HOPKINS, M.A., M.B., D.Sc., F.R.S.

THE NATURE OF ENZYME ACTION. By W. M. BAYLISS, D.Sc., F.R.S. 3s. net.

THE CHEMICAL CONSTITUTION OF THE PROTEINS. By R. H. ADERS PLIMMER, D.Sc. In Two Parts. Part I., 3s. net; Part II., 2s. 6d. net.

THE GENERAL CHARACTERS OF THE PROTEINS. By S. B. SCHRYVER, Ph.D., D.Sc. 2s. 6d. net.

THE VEGETABLE PROTEINS. By THOMAS B. OSBORNE, Ph.D. 3s. 6d. net.

THE SIMPLE CARBOHYDRATES AND THE GLUCOSIDES. By E. FRANKLAND ARMSTRONG, D.Sc., Ph.D.

THE POLYSACCHARIDES. By ARTHUR R. LING, F.I.C.

THE FATS. By J. B. LEATHES, D.Sc.

COLLOIDS. By W. B. HARDY, M.A., F.R.S.

ALCOHOLIC FERMENTATION. By A. HARDEN, D.Sc., F.R.S.

LONGMANS, GREEN, AND CO.,

LONDON, NEW YORK, BOMBAY, AND CALCUTTA.

THE SIMPLE CARBOHYDRATES AND THE GLUCOSIDES

BY

E. FRANKLAND ARMSTRONG, D.Sc., PH.D.

ASSOCIATE OF THE CITY AND GUILDS OF LONDON INSTITUTE

LONGMANS, GREEN, AND CO.

39 PATERNOSTER ROW, LONDON

NEW YORK, BOMBAY, AND CALCUTTA

1910

GENERAL PREFACE.

The subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single text-book upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult, in the case of the larger text-books, to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason, an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield full and independent information of the work which has been done upon the subject.

It has been decided as a general scheme that the volumes first issued shall deal with the pure chemistry of physiological products and with certain general aspects of the subject. Subsequent monographs will be devoted to such questions as the chemistry of special tissues and particular aspects of metabolism. So the series, if continued, will proceed from physiological chemistry to what may be now more properly termed chemical physiology. This will depend upon the success which the first series achieves, and upon the divisions of the subject which may be of interest at the time.

R. H. A. P.
F. G. H.

PREFACE.

TWENTY-EIGHT years ago the late Sir John Burdon Sanderson described one of the aims of Physiology as the acquirement of an exact knowledge of the chemical and physical processes of animal life. The recent history of physiological progress shows that investigations confined to the study of physical and chemical processes have been the most fruitful source of physiological advance, and it is principally the exact chemical study of the substances found in animals and plants which has enabled the physiologist to make this advance.

The last decade has seen very material progress in our knowledge of the carbohydrates, more particularly with regard to their inner structure, biochemical properties, and the mechanism of their metabolism. In consequence, many problems of the greatest fascination for the biochemist have presented themselves for solution.

This monograph aims at giving a summary of the present position of the chemistry of the carbohydrates. The reader is assumed to be already acquainted with the subject so far as it is dealt with in the ordinary text-books. The available information is, however, so widely scattered in the various scientific periodicals that it is impossible for any one approaching the subject to inform himself rapidly of what has been done. It is to meet such needs that this monograph is primarily intended.

A bibliography is appended, which contains references, classified under appropriate headings, to most of the recent works on the subject and to the more important of the older papers. It makes no claim to be exhaustive but serves to indicate how much is at present being done in this field.

E. F. A.

CONTENTS.

INTRODUCTION.

THE carbohydrates, together with the proteins, rank first in importance among organic compounds on account of the part they play, both in plants and animals, as structural elements, and in the maintenance of the functional activity of the organism.

The interest attaching to the group may be said to centre around glucose, this carbohydrate being the first to arise in the plant, and the unit group from which substances such as cane sugar, maltose, starch and cellulose are derived; it is also of primary importance in animal metabolism, as the main bulk of the carbohydrate in our food materials enters into circulation in the form of glucose.

Under natural conditions the higher carbohydrates are resolved into the simpler by the hydrolytic agency of enzymes, but these also exercise synthetic functions; the simpler carbohydrates are further resolved by processes which are undoubtedly akin to that of ordinary alcoholic fermentation. The carbohydrates are, therefore, of primary importance as furnishing material for the study of the processes of digestion and assimilation.

The carbohydrates are all remarkable on account of their optical characters; it is possible to correlate these with their structure. Of the large number of possible isomeric forms of the gluco-hexose $C_6H_{12}O_6$, sixteen in all, of which glucose is one, only three are met with in Nature, although twelve have already been prepared by artificial means; this natural limitation of the number produced in the plant and utilised by it and by the animal is a fact of great significance, and clear proof of the manifestation of a selective process at some period in the evolution of life. The elucidation of these peculiarities invests the inquiry into the nature and functions of the carbohydrates with peculiar interest and significance.

The simple carbohydrates are all of the empirical composition corresponding with the formula CH_2O, the most important being those containing five or six atoms of carbon. The members of the sugar group are usually distinguished by names having the suffix *ose*.

The simplest carbohydrate, CH_2O, formaldehyde or formal, is in all probability the first product of vital activity in the plant, the carbon dioxide absorbed from the air being converted into this substance

by the combined influence of sunlight and chlorophyll. The conversion of formaldehyde into glucose has been accomplished in the laboratory, but the transformation takes place in such a way that a variety of products is obtained; there is reason to suppose that but the single substance glucose is formed in the plant and that this is almost immediately converted into starch; in other words, the vital process is in some way a directed change. The record of the synthetic production of glucose and of the discovery of methods of producing the isomeric hexoses, as well as of determining the structure of the several isomerides, is one of the most fascinating chapters in the history of modern organic chemistry.

It would be impossible within the limits of a brief monograph to deal at length with the carbohydrates generally. In the following account, glucose will be taken as a typical sugar, and its properties and interrelationships will be considered more particularly with reference to their biochemical importance. The disaccharides and glucosides will be dealt with in a similar manner. Those who desire fuller information should consult the comprehensive works compiled by Lippmann and by Maquenne.

In discussing the various problems associated with the carbohydrates, the writer will strive to indicate the alternative views which have been advanced. He will, however, endeavour to develop the subject as far as possible as a logical whole, rather than leave the reader undecided at every turn. Such a method of treatment is more likely to stimulate inquiry by giving a picture of the present attitude of workers towards the various problems which the carbohydrates present.

CHAPTER I.

GLUCOSE (DEXTROGLUCOSE OR DEXTROSE).

It has been customary to speak of this sugar as *grape sugar*, to distinguish it from cane sugar, and on account of its occurrence in the juice of the grape and of other ripening fruits in association with fructose (laevulose); the two hexoses are probably derived from pre-existent cane sugar, as the three sugars are nearly always found together and cane sugar is easily resolved into glucose and fructose by hydrolysis :—

$$\underset{\text{Cane sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Fructose.}}{C_6H_{12}O_6}$$

Glucose is also formed from other more complex sugars when these are broken down by hydrolysis with the assistance of the appropriate enzymes or of acids—for example, from milk sugar or lactose, malt sugar or maltose, starch and cellulose. It is easily prepared from starch by the action of diluted sulphuric acid and is therefore to be purchased at small cost. It separates from an aqueous solution with a molecule of water of crystallisation, but this is held only loosely, as the anhydrous substance may be crystallised from dilute alcohol. Unlike cane sugar, it never separates in well-defined clear crystals from either water or alcohol, but is usually met with as crystalline powder.

Constitution of Glucose.—Glucose is represented by the molecular formula $C_6H_{12}O_6$. Five of the six atoms of oxygen are to be regarded as present in the alcoholic form, as hydroxyl (OH); the sixth under certain conditions manifests aldehydic functions. Thus, when acted upon by metallic hydroxides, glucose forms compounds which resemble the "alcoholates"; and it is converted by acids, acid oxides and chlorides, into ethereal salts or esters such as the following :—

$C_6H_7O(NO_3)_5$	$C_6H_7O(O \cdot CO \cdot CH_3)_5$	$C_6H_7O(O \cdot CO \cdot C_6H_5)_5$
Glucose pentanitrate.	Glucose pentacetate.	Glucose pentabenzoate.

On reduction, it takes up two atoms of hydrogen and is converted into a hexahydric alcohol; on oxidation it yields the monobasic acid, gluconic acid, $C_5H_6(OH)_5 \cdot CO \cdot OH$; when heated with a concentrated solution of hydrogen iodide, it loses the whole of its oxygen and is converted into an iodohexane $C_6H_{13}I$, which itself is a derivative of normal hexane, $CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_3$.

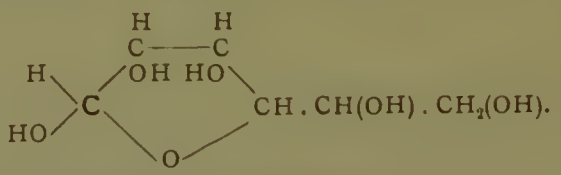
On account of the stability of glucose, it is to be assumed that each

hydroxyl group is associated with a different carbon atom; as glucose is a derivative of *normal* hexane, the constitutional formula of the aldehydic form may be written in the following manner:—

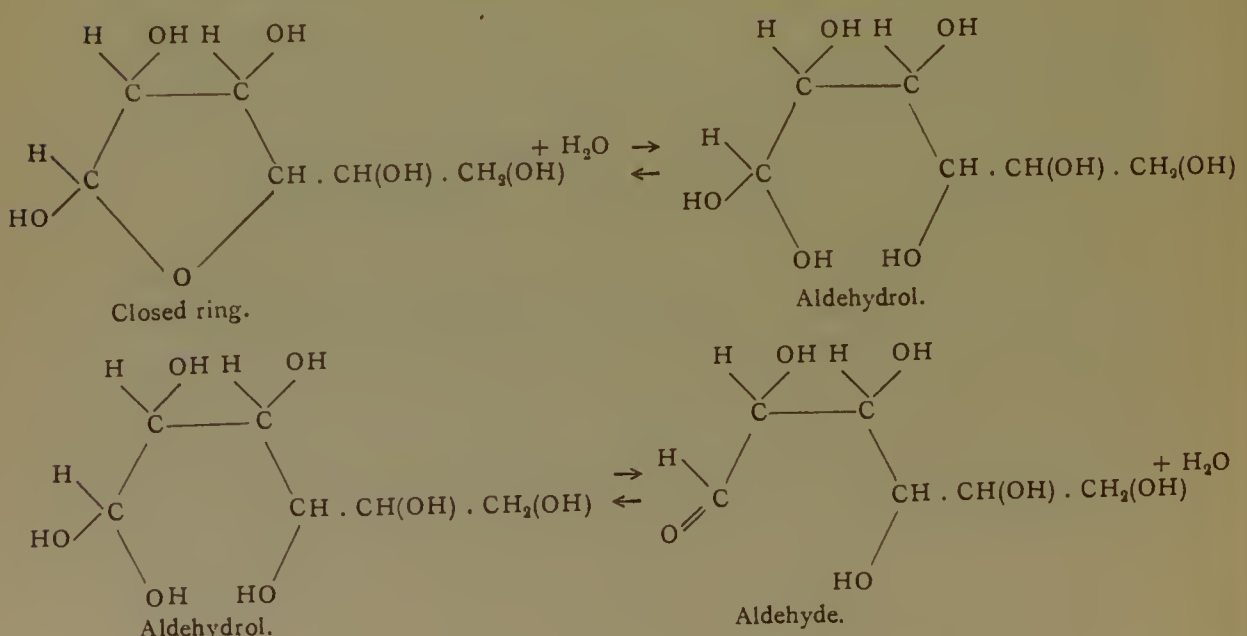
$$CH_2(OH) . CH(OH) . CH(OH) . CH(OH) . CH(OH) . CHO$$

But it was long a matter of remark that glucose, as a rule, is far less active than was to be expected, assuming it to be an hydroxyaldehyde. The difficulty was removed when Tollens, in 1883, proposed to represent it by a formula in which four of the carbon atoms are included in a ring, together with a single oxygen atom.

If the regular tetrahedron be adopted as the model of the carbon atom, and it be supposed that the four affinities are directed towards its four solid angles from the centre of a sphere within which the tetrahedron is inscribed, the direction of the affinities is such (109° 24′) that on uniting four such tetrahedra together, interposing as representative of the oxygen atom a ball with two affinities arranged in about the same directions as the two carbon affinities, a closed system or ring is formed almost naturally in which there is no strain, the internal angles being practically those in a regular pentagon, thus :—



This symbol has been very widely adopted, as it is in general accordance with the interactions of glucose. The behaviour of glucose as an aldehyde is accounted for if it be assumed that, when the ring is ruptured by hydrolysis, the closed-chain form passes into the aldehydic form in the following manner :—



This action being reversible, it is to be supposed that when an agent such as phenylhydrazine,[1] which will act upon aldehyde, is added to the aqueous solution, the small amount of aldehydrol present is attacked and removed; the equilibrium is thereby disturbed, but is rapidly restored by the formation of a fresh quantity of the aldehydrol, which in turn disappears but only to have its place taken by a further quantity. Ultimately the whole becomes converted into the aldehydic derivative.

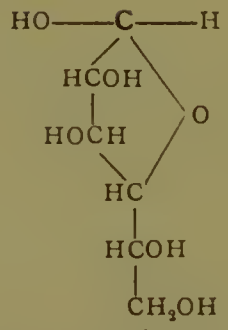
In considering the structure of glucose, the compounds which deserve attention in the first place are the two isomeric methyl-glucosides (α and β), which are formed by the interaction of glucose and methylic alcohol under the influence of hydrogen chloride. These compounds are the prototypes of the natural glucosides. They were discovered by Emil Fischer in 1893. He prepared them by dissolving glucose in cold methylic alcohol, saturated with dry hydrogen chloride gas. After several hours, when it had lost all cupric reducing power, the mixture was neutralised with lead carbonate. Crystals of the α-compound were obtained on concentrating the solution; the β-compound was isolated later from the mother liquor, and was first obtained crystalline by Van Ekenstein.

The methyl-glucosides differ considerably from glucose, more particularly in never behaving as aldehydes; and their rotatory power in solution is the same in a freshly-prepared solution as it is in one which has been kept for some time, which is not the case with glucose. They are undoubtedly formed by the introduction of methyl, in place of an atom of hydrogen, in the hydroxyl group attached to the carbon atom which exercises aldehydic functions in the open-chain form of glucose (glucose-aldehydrol). It is to be noted that the introduction of methyl in this position has the effect of rendering the ring far more stable than it is in glucose, as it is to be supposed that compounds such as phenylhydrazine, and oxidising agents such as Fehling's solution, are without action because the glucosides do not undergo hydrolysis in solution in the way that glucose does.

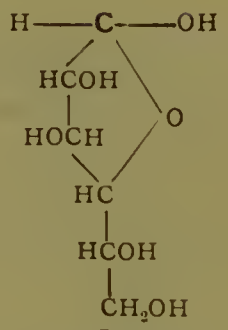
On reference to the closed-chain formula of glucose, it will be seen that the potentially aldehydic carbon atom (printed in clarendon type), as well as the three other carbon atoms in the ring, and also the atom which is immediately contiguous to the ring on the right-hand side of the formula (page 4), are all *asymmetric*, in the sense that each of them is associated with four different radicles. *Consequently the*

[1] See Chapter II.

closed-chain form of glucose may be written in either of two ways, thus :—



α-Glucose.

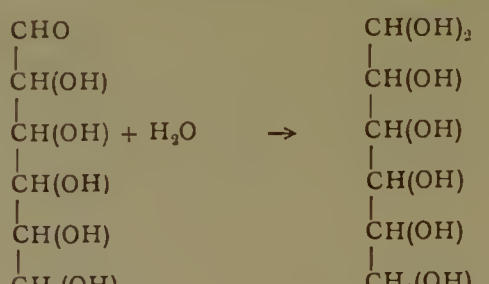


β-Glucose.

The two methylglucosides are to be regarded as the methyl derivatives of these two *stereoisomeric* forms of glucose.

Mutarotation—The Isomeric Forms of Glucose.—The hypothesis, that there are two stereoisomeric forms of glucose, is the only one hitherto proposed which affords a satisfactory explanation of a peculiar property, characteristic of glucose and other sugars manifesting aldehydic functions, now known as *mutarotation* or *multirotation* (but formerly termed *birotation*) ; namely, the optical rotatory power of the freshly dissolved substance changes gradually, sometimes increasing, but more usually falling, until a constant value is reached. The term *birotation* was introduced because the rotatory power of glucose in solution is about twice as great when it is freshly dissolved as that which it eventually assumes. The change takes place very slowly when highly purified materials are used, but almost immediately if a small quantity of alkali be added. The phenomenon was first observed by Dubrunfaut in 1846, and ascribed by him to purely physical causes. The subject has of recent years caused a good deal of controversy, and it is simplest to deal with the views that have been advanced in historical sequence.

E. Fischer, in 1890, noticed that the optical rotatory power of certain lactones closely related to the sugars underwent change in solution as the lactone became hydrolysed to the corresponding acid. He therefore ascribed the change which occurs with glucose to a like addition of a water molecule, and assumed that the glucose (aldehyde) underwent conversion into a heptahydric alcohol (aldehydrol) of lower rotatory power :—



Glucose (aldehyde). Alcohol (aldehydrol).

The subject assumed a new aspect when it was shown by Tanret, in 1896, that besides the anhydrous and hydrated forms of glucose other isomeric anhydrous modifications could be obtained. He described an *α*-glucose ($[\alpha]_D + 110°$), the initial rotatory power of which fell gradually to $[\alpha]_D + 52{\cdot}5°$; further, a *β*-glucose[1] of low initial rotatory power ($[\alpha]_D + 19°$), increasing to $[\alpha]_D + 52{\cdot}5°$ in solution; and, lastly, a *γ*-glucose ($[\alpha]_D + 52{\cdot}5°$) of unalterable rotatory power in solution. The three supposed isomerides were isolated by allowing glucose solutions to crystallise under different conditions—*α*-glucose separated at ordinary temperatures from solutions in 70 per cent. alcohol, and *β*-glucose from aqueous solutions at temperatures above 98° C.; *γ*-glucose was obtained by precipitating a concentrated aqueous solution of glucose with alcohol. *α*-glucose hydrate crystallises from aqueous solutions at the ordinary temperature. When powdered anhydrous glucose is added to water, it immediately undergoes hydration before passing into solution.

The behaviour of these isomeric forms does not fit in with the theory that the mutarotation is due to the conversion of an aldehyde into an aldehydrol; moreover, the increase in rotatory power from *β*- to *γ*-glucose has also to be explained.

Tanret, Lippmann and others suggested that some forms of glucose have a closed-ring structure, as proposed by Tollens, and that in solution these are completely converted into the isomeric aldehyde.

A more fruitful suggestion was made by Simon who drew attention to the optical behaviour of *α*- and *β*-glucose in relation to that of the isomeric methyl glucosides of which the structure was known:—

	$[\alpha]_D$		$[\alpha]_D$
α-Methyl-glucoside	+ 157°	*α*-Glucose	+ 105°
β-Methyl-glucoside	− 33°	*β*-Glucose	+ 22°

He suggested that the *α*- and *β*-glucoses are homologues of the *α*- and *β*-methyl glucosides, and that *both* contain a closed oxygenated ring.

Direct proof of the glucosidic structure of *α*- and *β*-glucose was afforded by their preparation from the corresponding glucosides effected by the writer. Both glucosides are resolved into methyl alcohol and glucose by appropriate enzymes, and as the enzymes condition the hydrolysis more quickly than the glucose which is formed can undergo isomeric change, it is possible to determine the nature of the sugar which is formed initially. In practice, this is done by preparing a clear solution of glucoside and enzyme, allowing hydrolysis to proceed

[1] Tanret actually termed the substance represented above as *β*-glucose *γ*-glucose and designated *γ*-glucose as *β*-glucose. The terms have been altered to bring them into agreement with the nomenclature now adopted.

for a short time and then observing the optical rotatory power of the solution before and after the addition of a drop of ammonia, which hastens the rate of the isomeric change, and therefore has the effect of establishing equilibrium almost immediately. As a glucose of high initial rotatory power was obtained from *α*-methyl glucoside, and one of low initial rotatory power from the *β*-glucoside, it is clear that *α*- and *β*-glucose correspond respectively to the *α*- and *β*-glucoside.

It remains to establish the nature of Tanret's *γ*-glucose, which he, as well as Simon and Lippmann, regarded as a third isomeride, ascribing the mutarotation of *α*- and *β*-glucose to their complete conversion into the isomeric aldehyde.

The change in rotatory power of glucose was shown to be a process of reversible isomeric change by Lowry in 1899. Lowry subsequently (1903) concluded that not only are *α*- and *β*-glucoses isodynamic compounds, but that Tanret's *γ*-glucose is a mixture in which these two compounds are present in equilibrium.

On concentration of the solution of such an equilibrated mixture, a point is reached when one of the constituents crystallises out from the saturated liquid. The mixture in solution is consequently thrown out of equilibrium; but as this happens a change takes place spontaneously to restore the equilibrium—*β* passing into *α*, or *vice versâ*. A solution of glucose containing *α*- and *β*-forms can therefore be made to yield wholly *α*- or wholly *β*-glucose on concentration, according to the temperature at which crystallisation takes place. The *α*-form, which is then the less soluble, is that obtained at lower temperatures; but above 98°, the *β*-form, being the less soluble at the higher temperature, alone separates. Were the change into aldehyde complete, as Simon and Lippmann suggest, it would be impossible by mere crystallisation to convert this into *α*-glucose.

Tanret (1905) has accepted the conclusion that there are but two isomerides of glucose, corresponding to the *α*- and *β*-methyl glucosides, and that his supposed third modification is an equilibrated mixture of these two forms. He has calculated from the rotatory power $[\alpha]_D + 110°$ of the pure *α*- and $[\alpha]_D + 19°$ of the pure *β*-form that the proportion in which these are in equilibrium is $\alpha = 37$ per cent., $\beta = 63$ per cent. in a 10 per cent. solution, and $\alpha = 40$, $\beta = 60$ per cent. in a concentrated aqueous solution.

By means of solubility determinations Lowry finds 52 per cent. of the *α*-form to be present in saturated solutions of glucose in methyl alcohol: the proportion of *α* decreases as the amount of water increases, amounting to 40 per cent. in the mixture $EtOH + H_2O$. He does not,

however, interpret the remaining 60 per cent. of sugar present in solution as β-glucose, but considers that some quantity of the aldehyde form is also present. (See also page 17.)

Stereoisomerism of the Aldohexoses.—A compound represented by the empirical formula,

$$CH_2(OH).CH(OH).CH(OH).CH(OH).CH(OH).CHO,$$

containing four asymmetric carbon atoms, should, according to the Le Bel-Van't Hoff hypothesis, be capable of existing in sixteen stereoisomeric forms, eight of which would be mirror images of the other eight and of equal but opposite rotatory power.

Thus, corresponding to ordinary dextroglucose (*d*-glucose), there should be a laevorotatory isomeride (*l*-glucose) of equal and opposite rotatory power, of like configuration but having the dissimilar radicles in reversed order.[1] In point of fact, when glucose is prepared by artificial means, a mixture in equal proportions of *d*- and *l*-forms is actually obtained. Such a mixture is optically inactive—whether the two forms actually combine or merely neutralise one another is unknown.

Although only three aldohexoses occur naturally (glucose, mannose, galactose), twelve of the sixteen possible isomerides are now known. Emil Fischer, to whom we owe the discovery of this remarkable series, has not only shown how they may be prepared, but has made them in such ways that their structural relationship may be regarded as established. His results are summarised in the following table :—

TABLE I.

ALDOHEXOSES.

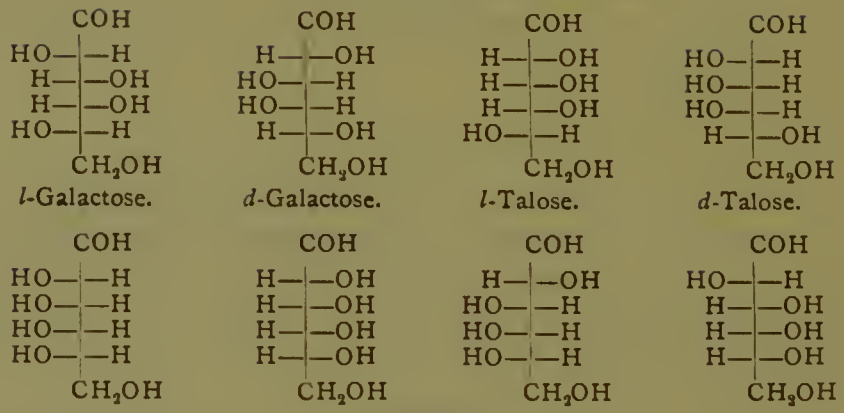
(*a*) *Mannitol Series.*

COH H—C—OH H—C—OH HO—C—H HO—C—H CH_2OH *l*-Mannose.	COH HO—C—H HO—C—H H—C—OH H—C—OH CH_2OH *d*-Mannose.	COH HO—C—H H—C—OH HO—C—H HO—C—H CH_2OH *l*-Glucose.	COH H—C—OH HO—C—H H—C—OH H—C—OH CH_2OH *d*-Glucose.
COH HO—C—H H—C—OH HO—C—H H—C—OH CH_2OH *l*-Idose.	COH H—C—OH HO—C—H H—C—OH HO—C—H CH_2OH *d*-Idose.	COH H—C—OH H—C—OH HO—C—H H—C—OH CH_2OH *l*-Gulose.	COH HO—C—H HO—C—H H—C—OH HO—C—H CH_2OH *d*-Gulose.

[1] The formulæ assigned to *d*- and *l*-glucose are chosen arbitrarily; that is to say, it is assumed that in the *d*-form the groups occupy a certain position, whence it follows that in the stereoisomeride they are present in the reversed position. For proof of the validity of the formulæ and the arguments by which they are deduced, the reader is referred to Fischer's summary in the *Berichte der deutschen chemischen Gesellschaft* for 1894 (p. 3189) and to the larger text-books on organic chemistry.

TABLE I. (*continued*).

(*b*) *Dulcitol Series.*



All unknown.

As two closed-chain forms should exist corresponding to each of the open chain aldehydic forms, no less a number of isomeric "glucoses" is foreseen by theory than 16 + 32 = 48.

Many of the other sugars exist in more than one form and show mutarotation. The available data are collected in the following table. The rotations given for the β-forms are the extremes at present recorded; probably in most cases they apply to products which are not entirely free from admixture with the α-isomeride.

TABLE II.

Sugar.	α-Form.	β-Form.	Equilibrated mixture.
d-Glucose	+ 110°	+ 19°	+ 52·5°
d-Galactose	+ 118°	+ 53°	+ 81°
d-Mannose	− ?	+ 23·5°	+ 14°
d-Fructose	− 104°	—	− 92°
l-Arabinose	+ 175°	—	+ 104°
l-Xylose	+ 79°	—	+ 19°
Rhamnose	− 17°	+ 31·5°	+ 9°
Maltose	+ 114°	—	+ 137°
Lactose	+ 86°	+ 35°	+ 55·3°
Tetramethyl glucose	+ 101°	+ 73·5°	+ 83·3°

The More Important Derivatives of Glucose.—The experimental work of the last ten years has shown that most of the derivatives of glucose likewise exist in two forms differing in physical properties, more particularly crystalline form, optical rotatory power and melting-point. The chemical behaviour of all these substances is such that it must be assumed that the aldehydic function has disappeared giving rise to the closed-ring structure already formulated.

Methyl Glucosides.—The methyl glucosides are of so much importance as the types, not only of glucosides generally, but of the two isomeric series of glucose derivatives, that it is essential to consider their behaviour at some length, and the reader is advised to familiarise himself with them.

The two glucosides are distinguished by the prefixes *a* and *β*, their properties being as follows :—

	Melting-point.	Rotatory Power.
a-Methyl glucoside	165°	+ 157°
β-Methyl glucoside	104°	− 33°

They are both colourless crystalline substances, the *a*-isomeride crystallising usually in long needles, the *β*-isomeride in rectangular prisms.

When hydrolysed by acids they yield methyl alcohol and glucose. At ordinary temperatures hydrolysis, even by moderately strong mineral acids, proceeds but slowly ; and if it be desired to study the course of hydrolysis it is advisable to work at elevated temperatures, say 70° to 80° C. As in other chemical reactions, the hydrolytic power of acids towards glucosides increases with a rise in temperature. A convenient method of experimenting consists in mixing acid and glucoside in a closed flask immersed in a thermostat so as to maintain the required temperature. Samples of the liquid are withdrawn at stated intervals of time, rapidly cooled by immersion in ice water to check hydrolysis, and the amount of glucose formed estimated either gravimetrically or with the polarimeter. To prevent evaporation it is advisable to add a little paraffin wax to the mixture of glucoside and acid. Measurements made in this way show that a definite fraction of the glucoside present is hydrolysed in each unit of time, the course of change following what is known as the logarithmic curve. The *β*-compound is attacked more rapidly than the *a*. This point will be referred to again in Chapter VI.

The methyl glucosides are also hydrolysed by enzymes, but both isomerides are not hydrolysed by the same enzyme. In fact, the action of enzymes towards the glucosides is specific, and each form requires its own particular enzyme: *a*-methyl glucoside is hydrolysed by maltase; *β*-methyl glucoside by emulsin. The enzymes act at ordinary temperatures, preferably not above 37° C., and are far more active as hydrolytic agents than acids.

Returning to the preparation of the glucosides described on p. 5, it will be noted that both forms are produced simultaneously, the

a-isomeride predominating. When solid anhydrous glucose (*a*-glucose) is dissolved in dry methyl alcohol containing dry hydrogen chloride the first change is the rapid conversion into a mixture of *a*- and *β*-glucose in nearly equal parts. Each of these then undergoes etherification, but inasmuch as the *β*-methyl glucoside is hydrolysed by hydrogen chloride 1·8 times as fast as the *a*-methyl glucoside, the former may be supposed to be more rapidly etherified. The primary result is therefore a mixture of *a*- and *β*-methyl glucosides, in which the latter is slightly in excess. On standing, slow conversion of the *β*-methyl glucoside into the more stable *a*-isomeride takes place. The equilibrated mixture of the glucosides contains 77 per cent. of the *a*- and 23 per cent. of the *β*-isomeride. If, however, the solution be neutralised as soon as etherification is complete, and before the isomeric changes take place, and the solvent be removed, a mixture of the two glucosides in approximately equal quantities is obtained. These may be separated by fractional crystallisation.

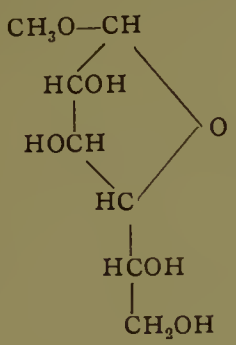
Such a process is somewhat tedious when *β*-methyl glucoside is the object of the preparation, and it is more convenient to make use of biological methods. On treatment with yeast, which contains the enzyme maltase, the *a*-methyl glucoside is hydrolysed to glucose and methyl alcohol, and the glucose is removed by fermentation, so that *β*-methyl glucoside, which is not attacked by yeast, alone remains, and can be isolated and purified.

When, on the other hand, *a*-methyl glucoside is desired, the action of the acid is allowed to continue until equilibrium is attained, and, after crystallisation of some quantity of the *a*-methyl glucoside, the mother liquors are again heated with a little acid. This has the effect of causing the *β*-glucoside present to be converted into *a*-glucoside until equilibrium is again reached, when 77 per cent. of the total solid present is *a*-glucoside, and in consequence a further quantity of *a*-glucoside crystallises on removal of the solvent.

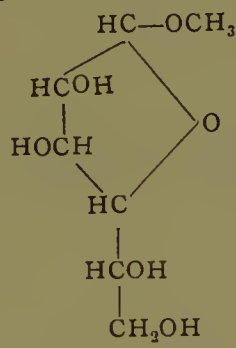
Fischer employs an alternative method, which consists in heating the alcoholic glucose solution with very little acid in an autoclave. It is then not necessary to neutralise before crystallisation of the *a*-glucoside.

Maquenne has prepared *β*-methyl glucoside by the action of methyl sulphate and sodium hydroxide on glucose dissolved in water. It is stated that the *β*-isomeride alone is formed under these conditions, but the quantity obtained is not large.

As already stated, the two methyl glucosides are regarded as stereo-isomeric γ-oxides,[1] and have the following structural formulæ :—



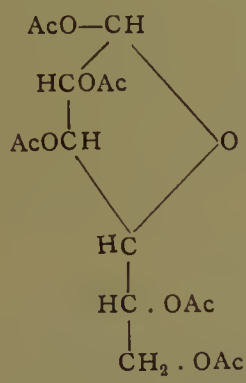
α-Methyl glucoside.



β-Methyl glucoside.

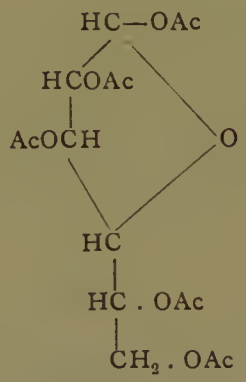
Glucose Pentacetates.—Under proper experimental conditions, all five hydroxyl groups in glucose become acetylated, the α- or β-pentacetate predominating in the product according to the method adopted. As these compounds form the starting-point for a number of syntheses, it is important to understand fully the methods of preparing them.

They have the following formulæ :—



α-Glucose pentacetate.

[Ac = C_2H_3O]



β-Glucose pentacetate.

To obtain the α-pentacetate it is necessary to acetylate glucose instantly before isomeric change can take place, since the presence of acid greatly accelerates the isomeric change from α- to β-glucose. This is done by adding anhydrous α-glucose to boiling acetic anhydride containing a small quantity of zinc chloride as catalyst. A violent action ensues, and the sugar passes into solution. The product is poured into water, which is changed from time to time to remove the acetic

[1] It is a characteristic property of γ-hydroxyacids to lose water very readily, forming ring compounds containing four atoms of carbon and one of oxygen : these are termed γ-lactones. Thus—

$$CH_2(OH) . CH_2 . CH_2 . CO_2H = H_2O + CH_2 . CH_2 . CH_2 . CO$$

γ-Hydroxybutyric acid. γ-Butyrolactone.

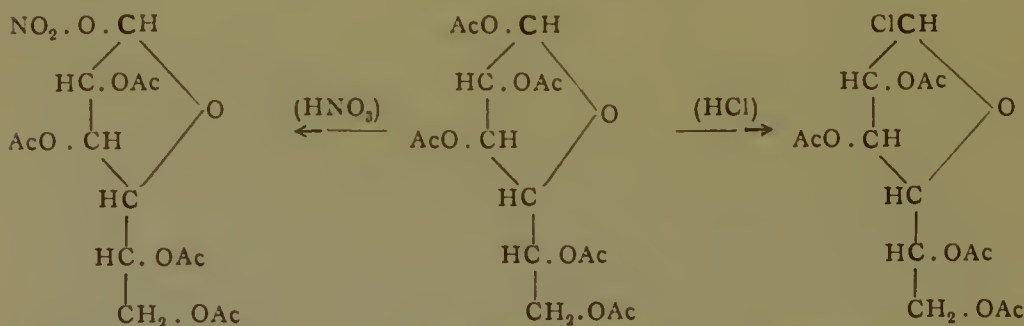
Similar four carbon-oxygen ring compounds when derived from γ-hydroxy compounds other than acids are named γ-oxides. The ring is termed a pentaphane ring.

acid ; finally the *a*-glucose pentacetate solidifies. The crude product contains both isomerides : it is purified by crystallisation from alcohol.

To obtain the *β*-pentacetate, glucose is mixed with acetic anhydride and sodium acetate, and heated for some time at the temperature of the water bath. As the change from *a*- to *β*-glucose in this case precedes acetylation, *β*-glucose pentacetate predominates in the final product, and may be separated by fractional crystallisation.

The pentacetates are colourless crystalline compounds, insoluble in water and readily hydrolysed by alkaline hydroxides. When heated with acetic anhydride either form is partially converted into the other : Jungius has shown that this change may also be effected by adding a small amount of sulphur trioxide to a solution of the acetate in chloroform.

Acetochloro, Acetonitro Glucoses.—In either isomeride, one of the acetyl groups—that attached to the terminal carbon atom (in Clarendon type) linked to the pentaphane oxygen atom—is far more active than the rest. When subjected to the action of anhydrous liquid hydrogen bromide or hydrogen chloride in sealed tubes at the ordinary temperature, this acetyl group alone is replaced by halogen. In this way *a*-pentacetyl glucose gives *a*-acetochloro-glucose, *β*-pentacetyl glucose the corresponding *β*-acetochloro-glucose—both beautifully crystalline colourless substances. Nitric acid acts in a similar manner causing the formation of crystalline *a*- and *β*-acetonitro-glucoses :—



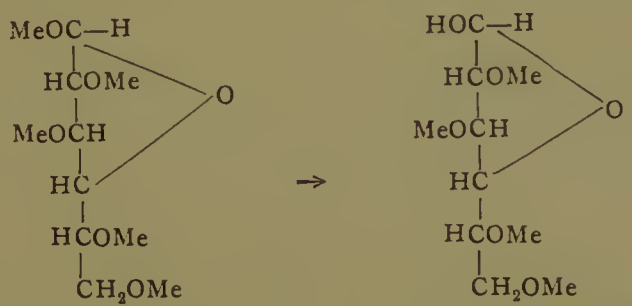
a-Acetonitro-glucose. *a*-Glucose pentacetate. *a*-Acetochloro-glucose.

Physical measurements also indicate that one of the acetyl groups is more easily detached than the others. This is proved by the fact that the rate at which the acetyl groups are removed by hydrolysis with alkali from the glucose pentacetates decreases as change proceeds ; yet the tetra-acetyl methyl glucosides, which contain four similarly placed acetyl groups but lack the one contiguous to the pentaphane oxygen, are hydrolysed by alkali at a rate which is constant throughout the whole change.

The chloro-, bromo- and nitro- groups are even more reactive than the acetyl group, and are easily replaced—for example, by methoxyl—on shaking a solution of the compound in anhydrous methyl alcohol with silver carbonate. The isomeric tetra-acetyl methyl glucosides thus obtained are converted, when hydrolysed by an alkali, into the corresponding isomeric methyl glucosides. These syntheses make it possible to pass from *α*-glucose to *α*-methyl glucoside through a series of *α*-compounds, and correlate all these compounds with *α*-glucose.

Methyl Glucoses.—The properties of the hydroxyl groups in glucose can be masked by their replacement by acetyl or benzoyl groups. The ethers so formed crystallise well, but the acid groups render these compounds resistant to the action of enzymes. The substitution of methoxyl for hydroxyl has a less disturbing influence; indeed methylation has little effect on the characteristic chemical reactions of reducing sugars except in increasing stability. The reducing sugars themselves cannot be directly methylated by any of the ordinary methods; but, as Purdie and Irvine have shown, it is possible to methylate the methyl glucosides by exhaustive treatment with methyl iodide and silver oxide. The products are purified by distillation in vacuum and subsequently obtained crystalline.

The isomeric *α*- and *β*-pentamethyl glucoses (*e.g.*, tetramethyl-methyl glucosides), when hydrolysed by acids, are converted into tetramethyl glucoses :—



α-Pentamethyl glucose. α-Tetramethyl glucose.

Both compounds yield finally the same tetramethyl glucose of constant rotatory power, but initially *α*- and *β*-tetramethyl glucoses are obtained from them, which exhibit mutarotation and slowly change in solution into the equilibrated mixture. Tetramethyl glucose is converted by Fischer's method of etherification into a mixture of *α*- and *β*-tetramethyl-methyl glucosides.

Tetramethyl glucose is not fermentable, but tetramethyl *β*-methyl glucoside is hydrolysed by emulsin, a fact which indicates that the introduction of the methyl groups into a glucoside does not put the resulting compounds out of harmony with enzymes.

A number of other sugars have been alkylated in like manner.

Anilides, Hydrazones, Oximes.—The interactions involved in the formation of anilides, hydrazones and oximes of glucose are most simply explained, on the assumption that the sugar is participating in a typical aldehyde reaction. None the less the occurrence of more than one form of all these derivatives forces the adoption of the closed-ring formula in such cases. Skraup early showed that a second phenyl hydrazone of glucose could be isolated, isomeric with that described originally by Fischer. Isomeric benzylphenyl hydrazones have also been obtained. The rotatory power of hydrazones changes in solution. It would go too far to discuss the nature of the isomerism here, nor is it yet satisfactorily established, but it may be pointed out that glucose-phenyl hydrazone may be formulated in syn- and anti-forms of the true aldehydic derivative, or as α- and β-hydrazides of γ-oxide structure, nor does this exhaust the possible isomerides.

Irvine and Moodie have shown in the case of tetramethyl glucose that both the oximes and anilides possess the γ-oxide ring in the hexose residue, and are thus to be regarded as derived from the α- or β-form of glucose, and not from an aldehydic isomeride. Their conclusions may reasonably be extended to the oximes and anilides of glucose, the latter of which Irvine and Gilmour have shown to exist in two modifications. The same authors failed to alkylate glucose phenyl hydrazone, or tetramethyl glucose phenyl hydrazone, and consider it still an open question whether these derivatives belong to the γ-oxide type.

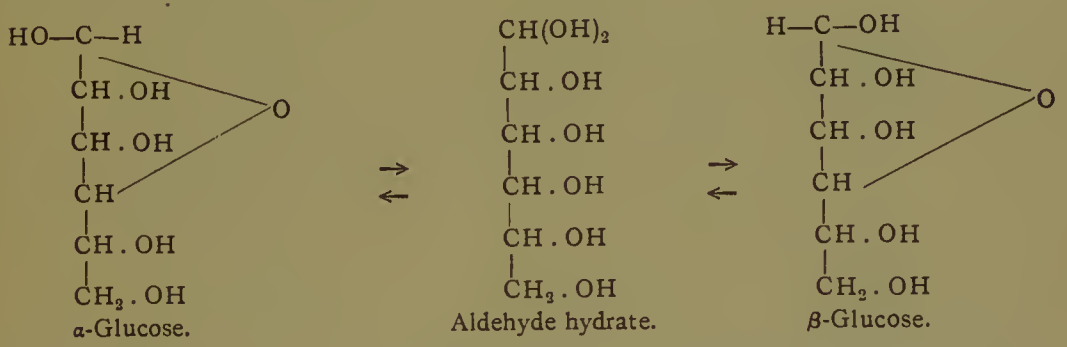
The properties of a number of these derivatives are summarised in the following table:—

TABLE III.

Glucose Derivative.	α-Series.		β-Series.	
	M.-pt.	$[\alpha]_D$.	M.-pt.	$[\alpha]_D$.
Penta-acetate	112°	+ 100°	134°	+ 3°
Acetochloro	63°	—	73°	+ 165°
Acetobromo	79°	—	88°	+ 198°
Acetonitro	92°	+ 1·5°	150°	+ 149°
Tetra-acetylmethyl . . .	100°	+ 137°	105°	− 23°
Methyl glucoside . . .	165°	+ 157°	104°	− 33°

Isomeric Change.—It remains to discuss very briefly the mechanism of the isomeric change $\alpha \rightleftarrows \beta$-glucose. Two rival explanations have been advanced which differ really only in one respect: Lowry considers the formation of the aldehyde or its hydrate, which involves the opening of the ring, to be an intermediate stage in the process; E. F. Armstrong, however, has formulated the change as taking place without any disruption of the γ-oxide ring.

According to Lowry's view, the change is represented by the scheme of equilibrium :—

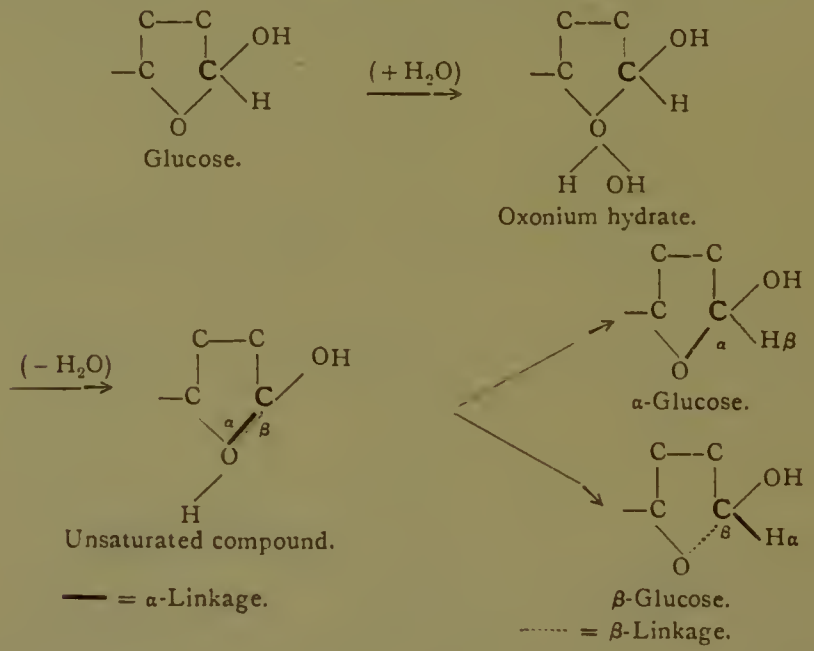


This scheme is intermediate in character between Fischer's view (p. 6), that mutarotation is due to hydration and the more recent view that mutarotation is due to isomeric change.

In anhydrous alcohol (which, however, contains traces of water) the velocity of the isomeric change $\alpha \rightleftarrows \beta$-glucose is small, but it increases as water is added and the opportunity for hydration is increased. Lowry takes the view that an aqueous solution of glucose contains a considerable proportion of aldehyde (open-chain form), in addition to α- and β-glucose (closed-ring forms), whereas in alcoholic solution there is little or no aldehyde.

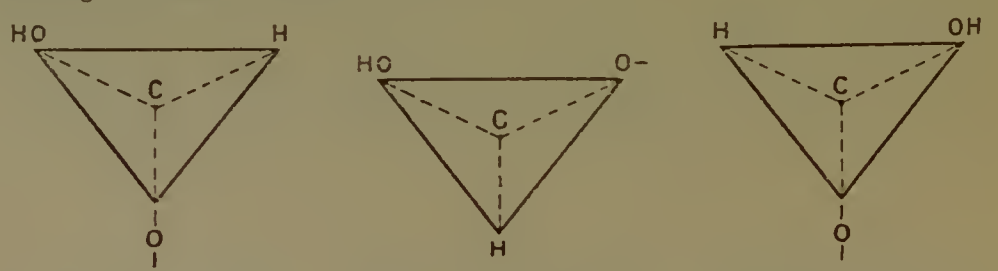
E. F. Armstrong considers the first stage in the process to be the formation, by the addition of water, of the oxonium hydrate, from which, by the elimination of water in another manner, an unsaturated compound results. It is possible to add the elements of water to this unsaturated bond in either of two ways, giving rise to the α- and β-glucoses respectively or their oxonium hydrates. Both isomerides are thus simultaneously formed. The stereoisomerism is pictured in this manner as arising from a difference in the position of the hydrogen atom relative to the pentaphane oxygen atom, both attached to the

terminal carbon atom [1] (in Clarendon type). In the following scheme only the carbon skeleton of the pentaphane ring is indicated :—



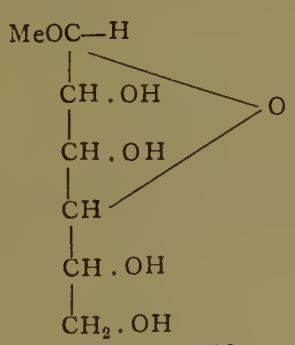
This explanation of the isomeric change has the advantage that it is equally applicable to the analogous interconversion of the *α*- and *β*-acetochloro glucoses observed in chloroform solution in presence of alkali, also to the interconversion of the *α*- and *β*-pentacetyl glucoses, neither of which can be explained on the aldehyde hydrate hypothesis ; and it also applies to the interconversion of the *α*- and *β*-methyl glucosides. In this last case Fischer has assumed that an intermediate compound of the acetal type is produced and the pentaphane ring is

[1] The asymmetric carbon atom in Clarendon type has attached to it the four radicles—(1) hydrogen, (2) hydroxyl, (3) the pentaphane oxygen, (4) a carbon atom of the ring. The stereoisomerism of α- and β-glucose is explained above as due to the interchange in the relative positions of the hydrogen and the pentaphane oxygen. This relationship is awkward to picture in plane formulæ; it is therefore more convenient to represent the stereoisomerism as due to the interchange in the relative positions of the hydrogen and hydroxyl radicles, as is done for example in the formula on previous pages. Reference to a solid model will show that this comes to exactly the same in the end, as the carbon atom in engaging with the pentaphane oxygen in its α or β position is necessarily rotated, so that a projection of the solid tetrahedron viewed in plan will show hydrogen alternately on the right and left of hydroxyl.

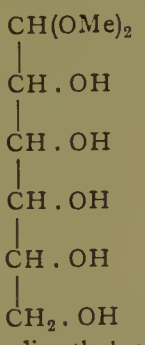


opened—a scheme identical with that just described as subsequently advocated by Lowry.

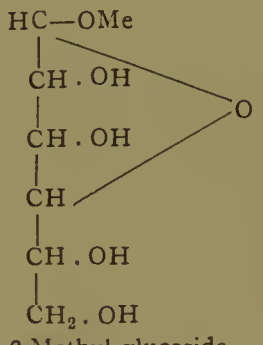
The first product of the action of dry methyl alcohol containing 1 per cent. of hydrogen chloride on glucose at the ordinary temperature is a syrup differing from either glucoside. This could not be analysed, but was regarded by Fischer as glucose dimethylacetal.[1] On heating this, it is in part converted into a mixture of the two glucosides in unequal quantities. A similar mixture is obtained when either glucoside is heated with the acidified alcohol.



α-Methyl glucoside.



Glucose dimethylacetal.



β-Methyl glucoside.

On the other hand, measurements of the velocity of their transformation made by Jungius led him to the conclusion that the two glucosides are directly convertible into each other, and that it is very improbable that an acetal is formed. Further, the reversible conversion of the α- and β-tetramethyl methyl glucosides takes place at temperatures of 110°-150° independently of the nature of the solvent used: a result which excludes the intermediate formation of a compound of an acetal type.

The isomeric change of one series of glucose derivatives into the other has been formulated in the foregoing on the hypothesis that additive oxonium compounds are formed in which the lactonic oxygen displays quadrivalency. Indeed no other explanation is applicable to all the transformations observed in the glucose series. Such additive oxonium compounds are well known to be formed in other cases, such as dimethylpyrone (Collie and Tickle). Recently Irvine and Moodie have brought forward evidence to show that tetramethyl glucose forms an oxonium derivative with isopropyl iodide. The presence of the etheric groups in the alkylated sugar apparently increases the basicity of the γ-oxidic oxygen atom, and so makes the identification of the oxonium compound possible.

From the biological point of view, the fact that glucose exists in

[1] A compound analogous to the acetal is obtained by the interaction of ethylmercaptan and glucose in presence of much hydrochloric acid. This is termed glucose ethylmercaptal,

$$CH_2(OH) . [CH(OH)]_4 . CH(SEt)_2.$$

It crystallises well, but cannot be converted into compounds analogous to the glucosides.

solution not as a single substance but as an equilibrated mixture of stereoisomeric γ-oxidic forms, readily convertible into one another, is of fundamental and far-reaching importance. If one of the stereoisomerides is preferentially metabolised in the plant or animal, in the course of either synthetic or analytic processes, the possibility of controlling the equilibrium in the one or other direction, so as to increase or limit the supply of this form, places a very delicate directive mechanism at the disposal of the organism. This question is undoubtedly one which demands the close attention of physiologists.

CHAPTER II.

THE CHEMICAL PROPERTIES OF GLUCOSE.

GLUCOSE, the other aldoses and the ketoses in general show a great tendency to become further oxidised; this is evidenced by their activity as reducing agents. They reduce alkaline copper solutions on warming forming red cuprous oxide, likewise ammoniacal silver solutions forming a metallic mirror. When heated with alkali, a sugar solution colours at first yellow, subsequently brown and finally decomposes: a variety of substances, including lactic acid and other hydroxy acids, are formed.[1] Valuable analytical methods for the estimation of glucose are based on the reaction with copper salts.

Particularly characteristic is the reaction of the sugars with excess of phenyl hydrazine on heating in dilute acetic acid solution. An orange-yellow insoluble phenyl osazone is formed, which serves to characterise glucose even when present only in very small quantities, though not to distinguish it from some of the isomeric hexoses which give the same or closely related phenyl osazones. The use of phenyl hydrazine possesses further a historical interest, as in the hands of Emil Fischer it served as one of the chief aids in the elucidation of the chemistry of the carbohydrates.

Glucose reacts with phenyl hydrazine in acid solution, acetic acid being usually employed, in two stages. In the first, which takes place in cold solution, a phenyl hydrazone is formed :—

$$C_6H_{12}O_6 + C_6H_5 . NH . NH_2 = C_5H_{11}O_5 . CH : N . NH . C_6H_5 + H_2O.$$

This is a colourless compound, soluble in water, and exists in two modifications, one or other of which is obtained according to the method of preparation (*cf.* p. 16).

The phenyl hydrazones of glucose and most of the other sugars, being easily soluble, are not adapted for characterising the parent

[1] The decomposition of the sugars by means of alkalis has been frequently studied of late years and the products formed compared with the hypothetical intermediate products of alcoholic fermentation. The subject, however, is still in too chaotic a state to repay discussion here. The decomposition, in particular of milk sugar, by lime to form the so-called saccharins (Kiliani) is also outside the range of this monograph.

sugars. An exception is afforded by mannose, which forms an almost insoluble phenyl hydrazone, and can thus be very readily detected. This compound affords a striking illustration of the influence exercised by the configuration of the molecule on its physical properties. Sparingly soluble phenyl hydrazones are also formed by the methyl pentoses.

Asymmetrically disubstituted hydrazines of the type, $NH_2 . NR . C_6H_5$, such as methylphenyl, benzylphenyl or diphenyl hydrazines, also react with the sugars, and some of these hydrazones are sparingly soluble and are characteristic of a particular sugar. Many of them are included in the following Table IV. In some instances two forms of the hydrazone have been described.

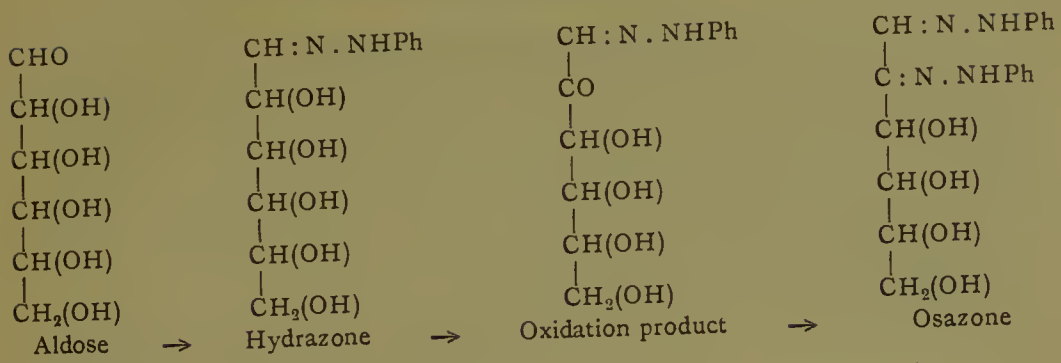
TABLE IV.

MELTING-POINTS OF SUGAR HYDRAZONES AND OSAZONES.

	Arabinose.	Glucose.	Mannose.	Galactose.	Maltose.	Lactose.
Hydrazones.						
Phenyl hydrazone	151°-153°	{115°-116° / 144°-146°}	186°-188°	150°	—	—
p-Bromophenyl hydrazone	150°	147°	208°-210°	168°	—	—
α-Methylphenyl hydrazone	161°	130°	178°	180°	—	—
α-Ethylphenyl hydrazone	153°	—	159°	169°	—	—
α-Amylphenyl hydrazone	120°	128°	134°	116°	—	123°
α-Allylphenyl hydrazone	145°	155°	142°	157°	—	132°
α-Benzoylphenyl hydrazone	170°	165°	165°	154°	—	128°
Diphenyl hydrazone	218°	161°	155°	157°	—	—
β-Naphthyl hydrazone	141°	—	157°	167°	176°	203°
Osazones.						
Phenyl osazone	160°	208°	208°	193°	206°	200°
p-Bromophenyl osazone	196°-200°	222°	—	—	198°	—
p-Nitrophenyl osazone	—	257°	—	—	261°	258°

To prepare the phenyl osazone glucose is heated with a considerable excess of phenyl hydrazine[1] (3-4 mols.) and acetic acid, the vessel being immersed in rapidly boiling water for an hour or more, when the insoluble osazone separates: it is best purified by crystallisation from a dilute solution of pyridine. The excess of phenyl hydrazine acts as an oxidising agent towards the phenyl hydrazone, converting the penultimate —CH(OH) group into —CO, and being itself reduced to aniline and ammonia. The CO group so formed interacts with a further molecule of phenyl hydrazine to form the osazone:—

[1] It is important that the phenyl hydrazine should be almost colourless and free from oxidation products.



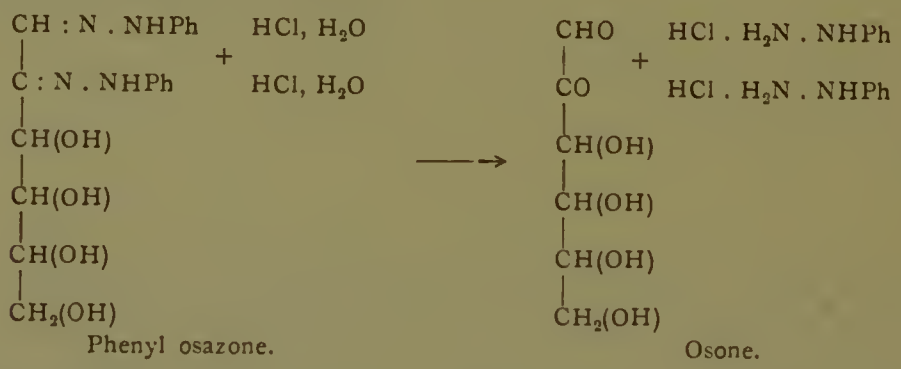
Glucose, mannose and fructose yield the same phenyl osazone. The osazones of the different sugars are as a class very similar in properties, those formed by the disaccharides being distinguished by their greater solubility in boiling water. The melting-points of the osazones depend very largely on the rate of heating and on the method of purification adopted, and too much dependence is not to be placed on them in identifying unknown sugars. Fischer, for example, states that carefully purified glucosazone heated rapidly in a narrow capillary tube begins to melt at 208° (corrected), and completely melts at this temperature with decomposition if the source of heat be withdrawn. When heating is continued at the same rate the thermometer rises to 213° before the glucosazone completely melts. When the heating is slower the substance begins to sinter and melt at 195°. In the case of the disaccharides, where the purification of the osazone is more difficult, the determination of the exact melting-point is even less reliable.

The asymmetrically disubstituted hydrazines do not form osazones with glucose on account of their being unable to act as oxidising agents. Fructose is more easily attacked by them, probably in consequence of the presence of the $CH_2(OH).CO$ group, and yields a methylphenyl osazone.

It is often a matter of considerable difficulty to obtain a carbohydrate in a pure state from solutions which may also contain inorganic salts or nitrogenous substances. One of the adopted methods is to isolate the phenyl hydrazone, purify this by crystallisation, and decompose it into sugar and phenyl hydrazine. Fischer originally used fuming hydrochloric acid to effect the decomposition. Benzaldehyde was substituted for this by Herzfeld; the phenyl hydrazone is boiled in water with a slight excess of benzaldehyde, and the phenyl hydrazine removed from solution as insoluble benzaldehyde phenyl hydrazone, $C_6H_{12}O_5 : N . NHPh + C_6H_5 . CHO = C_6H_{12}O_6 + C_6H_5CH : N . NHPh$. This method was repeatedly adopted with success by Fischer, but it gives less satisfactory results with the disubstituted hydrazones, in which case

formaldehyde may with advantage be substituted for benzaldehyde, as suggested by Ruff and Ollendorf. The hydrazone is dissolved in dilute formaldehyde and heated at the temperature of the water bath, $C_6H_{12}O_5 : N . NRR' + HCHO = C_6H_{12}O_6 + H . CH : N . NRR'$. The excess of formaldehyde is removed and the pure sugar solution concentrated in vacuum.

Fuming hydrochloric acid acts on the osazone in the same manner as it does on the hydrazone, eliminating in this instance both hydrazine groups to form an osone :—



Phenyl osazone. Osone.

Glucose, mannose and fructose, which form the same phenyl osazone, likewise form the same osone. These osones are colourless syrups; they act as strong reducing agents, and combine directly with phenyl hydrazine or with disubstituted phenyl hydrazines forming osazones. The osones combine also with *o*-phenylene diamine. They are not fermentable. On reduction glucosone is converted into fructose. This is the only method available of regenerating a sugar from the phenyl osazone. When the sugar originally used was an aldose the corresponding ketose results. The method is of great historical interest, as by its aid Fischer established the nature of the synthetical *a*-acrose. The osazones of the disaccharides are hydrolysed by acids to hexose, hexosone and phenylhydrazine—

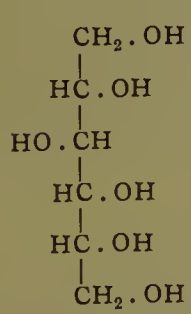
$$C_6H_{11}O_5 . O . C_6H_{10}O_4(N_2HPh)_2 + 2HCl + 3H_2O$$
$$= C_6H_{12}O_6 + C_6H_{10}O_6 + 2NH_2 . NHPh . HCl$$
Hexose. Hexosone.

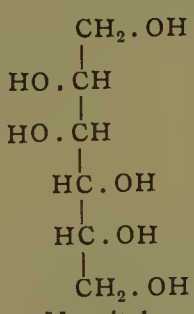
—and Fischer's hydrochloric acid method is thus not available for the conversion into osone. Since, however, the osazones of the disaccharides are soluble in boiling water, it is possible to remove the phenyl hydrazine residues by means of benzaldehyde (Fischer and Armstrong), and so obtain the osones—

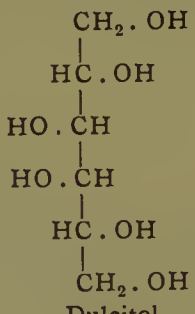
$$C_6H_{11}O_5 . O . CH_2 . [CH . OH]_3 . CO . CHO$$

These osones are similar to glucosone in properties: they are hydrolysed by enzymes in the same way as the parent disaccharides.

Reduction.—When reduced with sodium amalgam, glucose and its isomerides form the corresponding hexahydric alcohols, two hydrogen atoms being added to the hexose. Sorbitol is formed from glucose, mannitol from mannose and dulcitol from galactose. Fructose yields a mixture of the two alcohols, sorbitol and mannitol (see p. 40). These alcohols have the following configuration formulæ:—

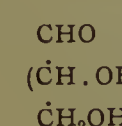

Sorbitol.

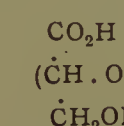

Mannitol.

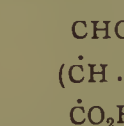

Dulcitol.

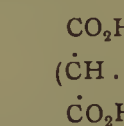
All three alcohols occur in plants, mannitol being widely distributed. In the fungi and some other orders mannitol exceeds glucose in quantity, or even replaces it. It has a sweet taste. None of the alcohols are fermented by yeasts; mannitol, however, is a product of some bacterial fermentations, and is attacked by many moulds and bacteria. Dulcitol, no doubt on account of the difference in configuration, is in general far more resistant to bacterial attack.

Oxidation.—Glucose gives rise to three acids, containing the same number of carbon atoms on oxidation; two of these acids are monobasic, the third is dibasic. Their structure is as follows:—


Glucose.


Gluconic acid.

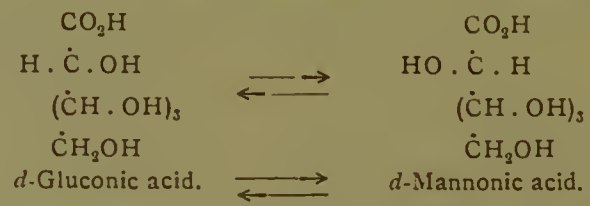

Glucuronic acid.


Saccharic acid.

In **gluconic acid** the aldehyde group of glucose is oxidised to carboxyl: it is conveniently prepared by the action of bromine on glucose. Gluconic acid in solution very readily passes over into a γ-lactone, the change, which is accompanied by an alteration in rotatory power, being a reversible one. Action is not complete, but continues until an equilibrium between acid and lactone is reached. Mannose and other aldoses form mannonic acid and similar acids corresponding to gluconic acid.

An important property of gluconic and similar acids, and one which has been of the utmost value in effecting the synthesis of the sugars, is their behaviour on heating with quinoline or pyridine. It is well known that in most substances containing an asymmetric carbon

atom, when they are heated, rearrangement takes place, so as to form the corresponding antimere mixed with the original substance. When gluconic acid is heated with quinoline or pyridine at 130°-150° it is partially converted into mannonic acid. The rearrangement is apparently restricted to the groups attached to the α-carbon atom, as is the case in the transformation of glucose to mannose by alkalis. It is reversible, mannonic acid being converted into gluconic acid:—



Similarly, *d*-galactonic and *d*-talonic acid are mutually interconvertible.

Saccharic acid is formed by the action of nitric acid on glucose; it forms a sparingly soluble acid potassium salt, which serves as a test for glucose. Saccharic acid is also produced from sucrose, raffinose, trehalose, dextrin and starch, all of which contain glucose. On the other hand, mucic acid—the corresponding oxidation product of galactose—is produced by the action of nitric acid on galactose, dulcitol, lactose, melibiose and the gums.

Glucuronic Acid.[1]—Physiologically the most interesting oxidation product of glucose is glucuronic acid, which is frequently found in the urine, combined with a variety of substances, forming compounds of glucosidic nature. It has not yet been identified as a plant product. Normally glucose is rapidly oxidised in the animal organism to carbon dioxide and water. When certain substances such as chloral or camphor, which are oxidised in the body only with difficulty, are brought into the system the organism has the power of combining them with glucose to form glucosides. In such compounds one end of the glucose molecule is shielded from attack, but oxidation takes place at the other extremity of the molecule, and a glucuronic acid derivative is formed. They are excreted in the urine. The faculty of removing injurious substances from circulation in combination with glucose seems to be common to both the animal and the vegetable kingdom, and the glucosides in the plant may be compared to the glucuronic acid derivatives in the animal. The glucuronates behave like glucosides, and form glucuronic acid when hydrolysed by mineral acids. The glucuronate most commonly employed for the preparation of the acid is euxanthic acid, a substance obtained in India from the urine of cows which have been fed with mango leaves. Euxanthic acid is very

[1] Also written Glycuronic acid.

readily hydrolysed by dilute acids and breaks down into euxanthon and glucuronic acid—

$$C_{19}H_{18}O_{11} = C_{13}H_8O_4 + C_6H_{10}O_7.$$

A number of substances when introduced into the organism are excreted in the urine as "paired" glucuronic acid compounds. The most important are included in the following list :—

isopropyl alcohol	chloral	benzene	turpentine oil
methylpropyl carbinol	butylchloral	nitrobenzene	camphor
methylhexyl carbinol	bromal	aniline	borneol
tertiary butyl alcohol	dichloracetone	phenol	menthol
tertiary amyl alcohol		resorcinol	pinene
pinacone		thymol	antipyrine
		α- and β-naphthol	etc.

As the formula indicates, glucuronic acid is the first reduction product of saccharic acid, and it was obtained in this way by Fischer and Piloty from saccharic acid lactone. Glucuronic acid forms a lactone which crystallises well. The paired acids are laevorotatory.

Synthesis and Degradation.—The methods devised in the laboratory for the formation of carbohydrates containing a greater or lesser number of carbon atoms than six in the chain are of interest.

The aldoses combine directly with hydrogen cyanide forming nitriles ; these, when hydrolysed, give rise to acids containing one carbon atom more than the original carbohydrate.

$$C_5H_{11}O_5.CHO + HCN = C_5H_{11}O_5.CH(OH).CN \rightarrow$$
$$C_5H_{11}O_5.CH(OH).CO_2H \rightarrow C_5H_{11}O_5.CH(OH).CHO$$

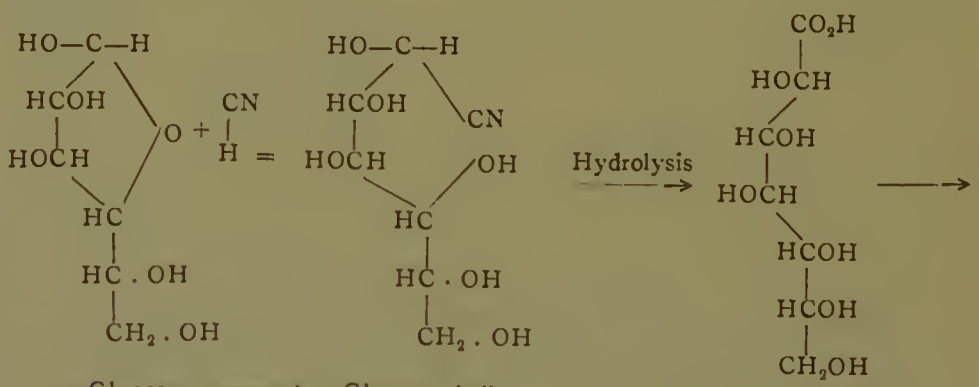
The lactones of these acids, when reduced with sodium amalgam, yield the corresponding aldoses with one carbon atom more than the original carbohydrate.

In this manner glucose can be obtained from arabinose, glucoheptose from glucose. The process has been continued by Fischer as far as the aldononoses in the case of glucose and mannose. It would be possible by such a method to advance step by step from formaldehyde to the higher sugars, but the operation would demand the expenditure of very large quantities of material.

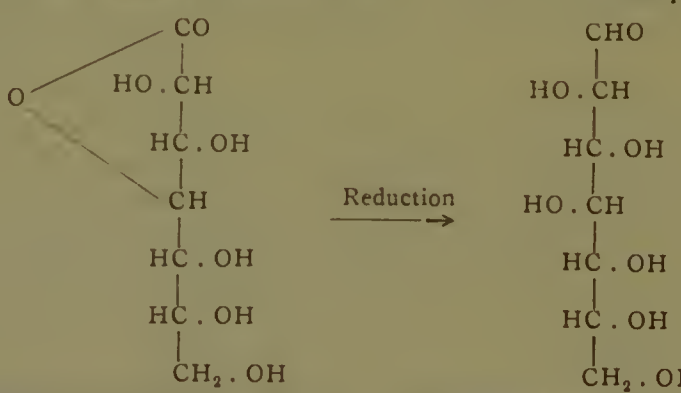
The cyanohydrin synthesis, however, is not in reality so simple as just pictured, inasmuch as usually two stereoisomeric nitriles are formed simultaneously. Arabinose gives both glucose and mannose, glucose yields two glucoheptoses. On the basis of the aldehydic formula for glucose a new asymmetric carbon atom is created in the nitrile, and, according to the ordinary rules, two forms will be produced unless the synthesis is asymmetric in character. Mannose affords the only instance at present recorded in which only one nitrile is formed.

An alternative view of the synthesis, based on the closed-ring

formula, considers the two nitriles as formed simultaneously from α- and β-glucoses by a process involving first the rupture of the γ-oxide ring, and secondly the addition of hydrogen cyanide. The presence of α- and β-glucose in unequal proportions and the probable difference in the rate of formation of the addition product in the two cases will explain the formation of the isomeric nitriles in unequal proportions. The various stages of the operation are formulated below in the case of the α-derivative—

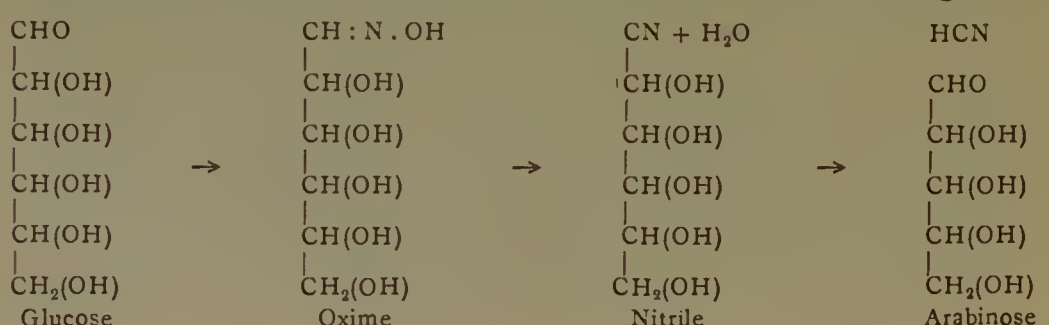


α-Glucose → α-Glucose nitrile → α-Glucoheptonic acid.



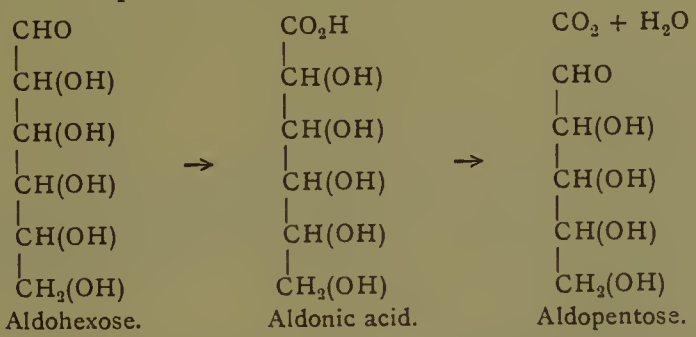
Lactone of α-Glucoheptonic acid. α-Glucoheptose, aldehyde formula.

The degradation of a sugar, *i.e.*, the conversion into one with fewer carbon atoms, has been studied by two experimental methods. In that of Wohl the oxime of glucose is heated with concentrated sodium hydroxide and converted into the nitrile of gluconic acid, from which, on further heating, hydrogen cyanide is eliminated and a pentose —*d*-arabinose—formed. The following scheme shows the changes :—



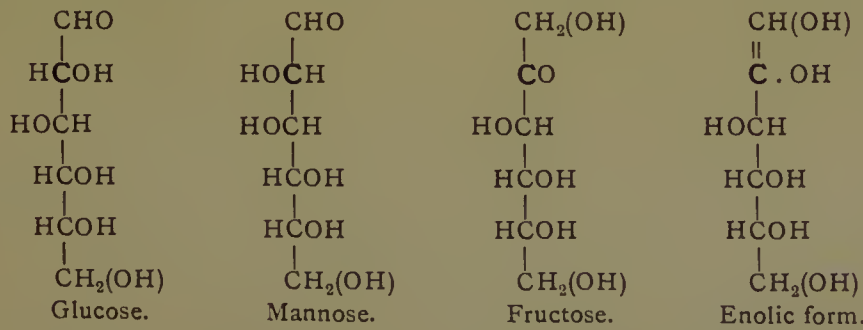
In practice it is preferable to heat the oxime with acetic anhydride and a grain of zinc chloride: a vigorous reaction ensues, and the pent-acetate of gluconic acid nitrile is formed from which hydrogen cyanide is eliminated by treatment with ammoniacal silver oxide.

The alternative method due to Ruff makes use of Fenton's mode of oxidation with hydrogen peroxide and ferrous salts. The aldose is first converted into aldonic acid, the calcium salt of which is subjected to oxidation, with the result that the carboxyl group is eliminated and a pentose formed.



Either of these methods is equally applicable to the conversion of a pentose into a tetrose, and by them it would be possible to pass from glucose to formaldehyde.

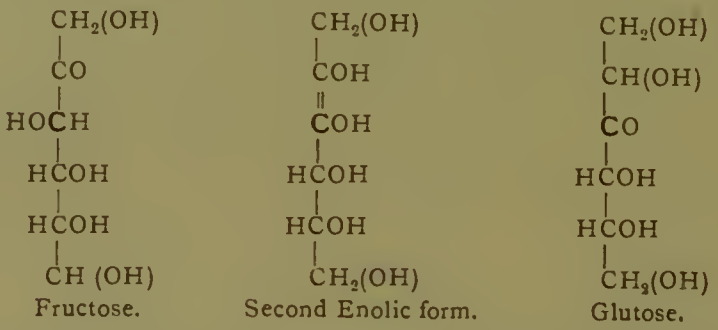
Interconversion of Glucose, Fructose and Mannose.—Glucose, fructose and mannose pass over into one another in aqueous solution in presence of alkalis. This most important transformation was first observed by Lobry de Bruyn and Van Ekenstein; it takes place slowly at ordinary temperatures, quickly and with much decomposition at higher temperatures. Starting from glucose, the optical rotation is observed to fall to about 0°; considerably more fructose than mannose is formed in the final product. The change was rightly explained by Wohl as due to conversion into the enolic (unsaturated) form common to all three carbohydrates:—



The sugar originally present is slowly transformed into enol; this is reconverted into all three of the possible hexoses. It is to be supposed that the formation of enol from each one of the hexoses and the

reverse changes all take place with different velocities ; the reaction is further complicated by secondary stages.

For example, fructose can give rise to a second enolic form, and this will occasion the formation of other isomerides, *e.g.*, glutose :—

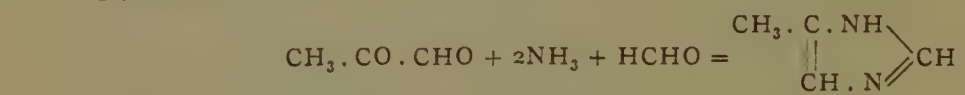


Fructose. Second Enolic form. Glutose.

which Lobry de Bruyn has isolated as a regular product of the transformation of glucose. The change is obviously exceedingly complicated. Prolonged action of the alkali or action at a high temperature leads to the formation of hydroxy acids.

The guanidine compounds of glucose, fructose and mannose show changes of rotatory power in aqueous solution due to the interconversion of the three hexoses brought about by the guanidine. The changes are very similar to those caused by alkalis, but fewer side reactions take place in the case of guanidine.

Since lactic acid and various hydroxy acids result from the action of alkalis on glucose (p. 21), the action of ammonia might cause the formation of alanine or other amino acids. Windaus and Knoop, in investigating this point, find that the strongly dissociated zinc hydroxide ammonia acts on glucose even in the cold, producing methyl glyoxaline, a closed-ring compound containing nitrogen. Amino acids are not formed. To explain this transformation, it is assumed that glyceric aldehyde is first formed, which passes into methyl glyoxal ; this in its turn is acted upon by ammonia and formaldehyde to give methyl glyoxaline :—



Windaus finds that the reaction is not confined to glucose, but that the same methyl glyoxaline is yielded by mannose, fructose, sorbose, arabinose, xylose and rhamnose, or by the disaccharide lactose.

***d*-Glucosamine.**—Glucosamine, or aminoglucose, is of interest as being the first well-defined carbohydrate compound isolated from an animal tissue (Ledderhose, 1878). It is obtained by boiling the shells of lobsters, particularly the claws, with concentrated hydrochloric acid.

The glucosamine hydrochloride so formed is a colourless crystalline compound. Lobster shell consists of carbonate of lime and a substance termed chitin, and yields acetic acid, besides glucosamine on hydrolysis. Chitin is stated by Offer to be a monoacetyl diglucosamine ; quite recently Irvine has established the identity of the chitins derived from various invertebrate animal structures. He considers chitin to contain acetylamino glucose and amino glucose residues in the proportion of three to one, in agreement with the formula $(C_{30}H_{50}O_{19}N_4)_n$.

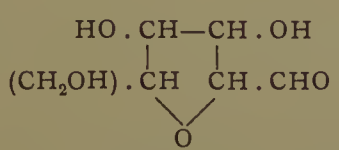
Glucosamine was obtained by Winterstein from fungus cellulose ; indeed chitin seems to be the most important cell-wall material of the fungi. Glucosamine is a constituent of the mucins and mucoids. It has the formula :—

$$CH_2OH \cdot \underset{OH}{C} \cdot \underset{OH}{C} \cdot \underset{H}{C} \cdot CH(NH_2) \cdot CHO$$

the stereochemical position of the amino group being as yet uncertain.

Glucosamine is prepared from the hydrochloride by decomposing it with diethylamine (Breuer) or sodium methoxide (Lobry de Bruyn). It derives special interest from the fact that it may be regarded as a link between the carbohydrates and the *a*-hydroxyamino acids. The synthesis of glucosamine, by Fischer and Leuchs, which at the same time established its constitution, thus becomes of enhanced importance. By the combination of *d*-arabinose and ammonium cyanide, or of *d*-arabinoseimine with hydrogen cyanide, *d*-glucosaminic acid was obtained and its lactone reduced to glucosamine. Glucosamine forms a penta-acetyl derivative and also an oxime, semi-carbazone and phenyl hydrazone, but it cannot be converted into glucose, though it gives glucose phenyl osazone when heated with phenyl hydrazine. Nitrous acid converts it into a compound $(C_6H_{10}O_5)$, formerly regarded as a sugar, and termed chitose : this forms chitonic acid when oxidised. Glucosamine is often regarded as a derivative of chitose, and termed chitosamine.

Chitose was shown by Fischer and Andreae to be a hydrated furfurane derivative rather than a true sugar, formed by simultaneous elimination of the amino group and anhydride formation. It has the formula :—



Isomeric with glucosamine is isoglucosamine, obtained by Fischer by reducing phenyl glucosazone. This has the formula :—

$$CH_2(OH) \cdot [CH(OH)]_3 \cdot CO \cdot CH_2 \cdot NH_2$$

Lobry de Bruyn has shown that glucosamine in aqueous solution changes to a substance which can be obtained more readily by the action of alcoholic ammonia on fructose. This substance yields a pyrazine derivative on oxidation (Stolte), and its formation from glucosamine would appear to take place according to the equation :—

$$2C_6H_{13}O_5N + O = C_{12}H_{20}O_8N_2 + 3H_2O.$$

The product, for which the name "fructosazine" is suggested, has been shown to be 2, 5 – ditetrahydroxy butylpyrazine.

But little is at present known of the amino derivatives of other carbohydrates.

CHAPTER III.

THE HEXOSES AND PENTOSES.

THE general properties of the monosaccharides have been fully dealt with in the foregoing and exemplified in the case of glucose. In dealing with the remaining hexoses it is only necessary to recapitulate briefly their more important properties and any salient points of difference from glucose.

Glucose and fructose are the only two of the monosaccharides which occur naturally. The others are found in nature as polymerides, or in the form of alcohols, and are prepared by hydrolysis or oxidation.

Fructose and sorbose are types of the ketohexoses, a group which has been much less investigated than the aldohexoses. Both fructose and sorbose have the ketonic oxygen attached to the α-carbon atom, but a number of other isomerides are possible in which the keto group is situated elsewhere in the molecule. The ketohexoses do not yield acids containing the same number of carbon atoms on oxidation, but the molecule breaks into two at the ketonic group.

TABLE V.—THE MONOSACCHARIDES.

	TRIOSES.	
Aldose	Glyceric aldehyde	
Ketose	Dioxyacetone	
	TETROSES.	
Aldoses	*d*- and *l*-Erythrose	
	d- and *l*-Threose	
	PENTOSES.	
Aldoses	*d*- and *l*-Arabinose	
	d- and *l*-Xylose	
	l-Ribose	
	l-Lyxose	
	METHYLPENTOSES.	
Aldoses	Rhamnose	
	Fucose, Rhodeose	
	Chinovose	
	HEXOSES.	
Aldoses	*Mannitol series*	*Dulcitol series*
	d- and *l*-Glucose	*d*- and *l*-Galactose
	d- and *l*-Mannose	*d*- and *l*-Talose
	d- and *l*-Gulose	
	d- and *l*-Idose	
Ketoses	Fructose	Tagatose
	Sorbose	
HEPTOSES.	OCTOSES.	NONOSES.
Mannoheptose	Mannooctose	Mannononose
Glucoheptose	Glucooctose	Gluconononose
Galactoheptose	Galactooctose	

Mannose.—*d*-Mannose[1] is widely distributed in nature in the form of anhydride-like condensation products termed mannosans which are converted into mannose when hydrolysed by acids; it does not occur in more simple form. A convenient source for its preparation is the vegetable ivory nut. Mannose is the true aldehyde of mannitol, and may be obtained from it by oxidation. It is of interest that it was first prepared by Fischer and Hirschberger in this manner, and only subsequently identified as a natural product. It is very similar to *d*-glucose in its general properties, exhibits mutarotation, and forms the same phenyl osazone as glucose and fructose. Mannose is altogether remarkable in forming a sparingly soluble phenyl hydrazone, which enables it to be very easily identified. This hydrazone is precipitated within a few minutes when phenyl hydrazine is added to a solution of mannose.

Mannose forms an additive compound with hydrogen cyanide which, on hydrolysis, yields mannoheptonic acid. Apparently one only of the two possible isomerides is formed. The mannoheptose obtained from this is very similar to mannose, and forms a sparingly soluble phenyl hydrazone. On reduction it yields the alcohol $C_7H_{16}O_7$ identical with the natural perseitol.

Galactose.—*d*-Galactose occurs as a constituent of milk sugar and raffinose, also in many gums and seaweeds as the polymeric form galactan; its presence in the form of a galactoside is very rare. It resembles glucose in properties; characteristic is the formation of mucic acid on oxidation with nitric acid, and this may be used for its identification. By the action of alkalis it is transformed into *d*-talose and *d*-tagatose. It is fermented by some yeasts, but not by all those which ferment glucose; a fact which has been taken as indicating that a special galacto-zymase is required for the fermentation.

α-Methyl galactoside is not hydrolysed by enzymes; *β*-methyl galactoside is attacked, like milk sugar, by the lactase of kephir, by the lactase present in some yeasts, and by a lactase present in an aqueous extract of almonds (see Chapter V.).

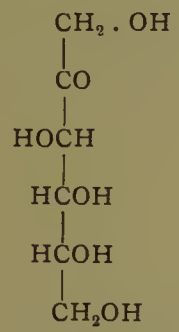
Under abnormal conditions galactose is formed in the sugar beet, and appears in combination with sucrose as the trisaccharide, raffinose. The quantity of raffinose is increased abnormally by disturbances of growth, such as those occasioned by sudden frost. Under these conditions the galactans are supposed to undergo hydrolysis and form galactose. Apparently the plant, when confronted with galactose, utilises it first to form a disaccharide, melibiose, composed of glucose and galactose, and then makes use of the glucose half in this di-

[1] For the configuration formula, see Table I., p. 9.

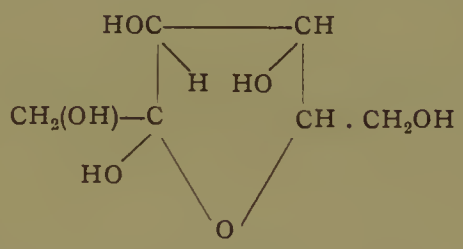
saccharide, according to its fixed habit, by combining it with fructose, with the result that a compound carbohydrate containing all three simple hexoses is formed.

Galactose is the sugar of the brain whence it was isolated and described under the name cerebrose by Thudichum. It is a constituent of the cerebrosides known as phrenosin and kerasin.

Fructose.—*d*-Fructose or Laevulose, discovered by Dubrunfaut in 1847, occurs together with glucose in the juices of fruits, etc., the mixture being often termed fruit sugar or invert sugar. Combined with glucose it occurs as cane sugar, raffinose, etc. The polysaccharide inulin yields fructose alone when hydrolysed. Fructose is a ketohexose of the following constitution :—



Fischer formula.



γ-Oxide formula.

Fructose crystallises less easily than glucose, and its derivatives are also difficult to crystallise. It exhibits mutarotation, and, like glucose, exists in solution presumably as an equilibrated mixture of stereoisomeric forms. It is remarkable for the very large change produced in the specific rotatory power by changes of temperature. The rotatory power falls (*i.e.*, becomes less negative) as the temperature is increased, and at 82° C. it is equal and opposite to that of glucose.

Fructose shows a number of characteristic reactions. Hydrogen bromide interacts with fructose in ethereal solution to form bromomethylfurfural $\begin{matrix}CH : C(CH_2Br)\\ CH : C(CHO)\end{matrix}\!\!>\!O$, a substance which crystallises in golden yellow rhombic prisms; the ethereal liquid is coloured an intense purple red (Fenton and Gostling). A β-oxy-γ-methylfurfural is produced on heating concentrated solutions of fructose under pressure, preferably with oxalic acid.

On prolonged boiling with dilute mineral acids laevulinic acid, $CH_3 . CO . CH_2 . CH_2 . CO_2H$, is formed together with formic acid and humus substances.

When oxidised by means of mercuric oxide fructose forms glycollic acid, $CH_2(OH) . CO_2H$, and trihydroxyglutaric acid, $CO_2H . (CH . OH)_3 . CO_2H$.

By the action of methyl alcohol and hydrogen chloride on fructose a syrup is obtained which probably represents a mixture of methyl fructosides. This syrup is partially hydrolysed by yeast extract, but, inasmuch as Pottevin has shown that it is not hydrolysed by *S. octosporus*, *Mucor mucedo* and other ferments which attack cane sugar and maltose, the hydrolysis is presumably caused by an enzyme other than invertase or maltase (see Chapter IV.).

Fructose, like glucose, forms an additive compound with hydrogen cyanide which yields fructose carboxylic acid on hydrolysis; this, when boiled with hydriodic acid, is converted into methyl butylacetic acid, $C_4H_9 . CHMe . CO_2H$. This reaction and the behaviour on oxidation establish the formula of fructose.

Fructose forms the same osazone as glucose; it also forms osazones with some disubstituted phenyl hydrazines, the primary $CH_2(OH)$ group being more easily oxidised by these than the secondary CH(OH) group in glucose.

Sorbose.—Sorbose was originally discovered by Pelouze in 1852 and was isolated from the juice of mountain ash berries which had been exposed to the air for many months. These berries contain the alcohol sorbitol, which, under the influence of an oxidising organism, shown by Emmerling to be identical with the bacterium xylinum of Adrian Brown, is oxidised to sorbose. The brilliant researches of Bertrand have given a complete explanation of the transformation, and have rendered the preparation of sorbose a relatively simple matter. Sorbose is a ketose having the formula :—

$$\begin{array}{l} CH_2OH \\ \dot{C}O \\ HO\dot{C}H \\ H\dot{C}OH \\ HO\dot{C}H \\ \dot{C}H_2OH \end{array}$$

It has a marked crystallising power, is not fermentable, and generally behaves as fructose; on reduction it yields sorbitol. Lobry de Bruyn has shown that under the influence of alkali *d*-sorbose is converted into *d*-gulose, *d*-idose and *l*-galactose, and so affords a connecting-link between hexoses of the mannitol and dulcitol series. This reaction is of importance, as the direct synthesis of a hexose of the dulcitol series has not been achieved.

The Pentoses, $C_5H_{10}O_5$.—Only two pentoses occur naturally, *l*-arabinose and *l*-xylose. These are widely distributed in the vegetable kingdom as polysaccharides of high molecular weight, the so-called pentosans, and are never found as the simple sugars. Xylose occurs in straw, arabinose in gums. In the animal kingdom pentoses form an important constituent of the nucleoproteins and nucleic acids. The pentosans are resistant towards alkali, and require prolonged heating with mineral acids to effect hydrolysis. No ferments are known which hydrolyse them. Inasmuch as the pentoses, in particular xylose, function essentially as skeletal, and not as food products in the plant, it is to be expected that they will be outside the range of plant ferments.

The eight possible aldopentoses are given in the following table, together with their configuration formulæ. The table also contains the remaining lower members of the group of monosaccharides, *viz.*, 4 tetroses and 2 trioses. Both the natural pentoses belong to the *l*-series. The *d*-isomeride of arabinose can be obtained from *d*-glucose by the degradation methods indicated in the previous chapter.

TABLE VI.

ALDOPENTOSES.

CHO HO|H HO|H HO|H CH_2OH *l*-Ribose.	CHO H|OH H|OH H|OH CH_2OH *d*-Ribose Unknown.	CHO H |OH HO| H H |OH CH_2OH *l*-Xylose.	CHO HO| H H |OH HO| H CH_2OH *d*-Xylose.
CHO H |OH HO| H HO| H CH_2OH *l*-Arabinose.	CHO H|OH H|OH HO| H CH_2OH *l*-Lyxose Unknown.	CHO HO| H H |OH H |OH CH_2OH *d*-Arabinose.	CHO HO| H HO| H H |OH CH_2OH *d*-Lyxose.

ALDOTETROSES.

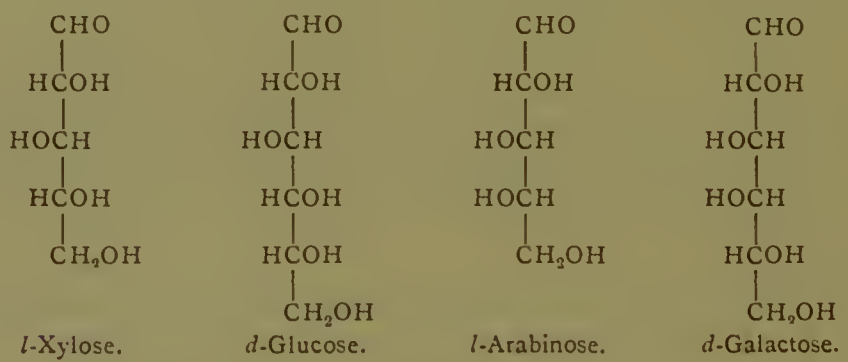
CHO HO|H HO|H CH_2OH *l*-Erythrose.	CHO H|OH H|OH CH_2OH *d*-Erythrose.	CHO H |OH OH| H CH_2OH *d*-Threose Unknown.	CHO HO| H H |OH CH_2OH *l*-Threose.

ALDOTRIOSE.

CHO HCOH CH_2OH	CHO HOCH CH_2OH

d- and *l*-Glycerose.

Although belonging to the *l*-series, the natural pentoses are in reality closely related to the natural hexoses. As the formulæ below show, the arrangement of the groups on the upper four carbon atoms is the same in each case in galactose and arabinose, and the same also in glucose as it is in xylose:—



l-Xylose. *d*-Glucose. *l*-Arabinose. *d*-Galactose.

In this connection, it is not without interest that some polysaccharides yield both xylose and glucose on hydrolysis, whilst arabinose and galactose occur together in many gums.

When the cyanohydrin synthesis is applied to natural *l*-arabinose a mixture of two nitriles is obtained, and the corresponding acids, when reduced, give rise to *l*-glucose and *l*-mannose; similarly, *l*-xylose can be converted into *l*-gulose and *l*-idose. *d*-Glucose, when degraded by the methods of Ruff or Wohl, gives *d*-arabinose; *d*-galactose forms *d*-lyxose. The carbon atom which requires to be eliminated in order that *d*-glucose may give rise to the natural *l*-xylose, a transformation which there is reason to think may take place in the plant, is not the one affected by the processes described, but is situated at the extreme end of the chain. No chemical means of effecting this change has as yet been discovered.

Arabinose and xylose show the usual aldose reactions. They are not fermented by yeasts. Arabinose forms a characteristic, almost insoluble, diphenyl hydrazone. Xylose is best recognised by conversion into xylonic acid, and isolation of this as the cadmium bromide double salt.

Pentoses are determined quantitatively by distillation with hydrochloric acid when furfural is formed. This is coupled with phloroglucinol, and the condensation product isolated and weighed. The colour reactions obtained on heating with orcinol or phloroglucinol and hydrochloric acid are very characteristic, and frequently used for detecting the pentoses.

Methyl Pentoses.—A class of carbohydrates, of which several representatives have been discovered latterly in plants, is the methyl pentoses. In these one of the hydrogen atoms in the primary alcohol group of the pentose is replaced by methyl. The biochemical significance of these compounds is not yet understood. They show most of the characteristic reactions of glucose, but are not fermentable by yeasts.

Rhamnose, $C_6H_{12}O_5$, is a constituent of many glucosides, the best known of which are quercitrin and xanthorhamnin. It occurs particularly in combination with flavone derivatives. Rhamnose crystallises with a molecule of water, the hydrate having the composition $C_6H_{14}O_6$. In consequence of this it was at one time regarded as belonging to the hexahydric alcohols and termed "isodulcitol". Rhamnose has the following configuration formula, in which the positions of the groups attached to one carbon atom are still uncertain :—

$$\begin{array}{l} \qquad\quad HO\ \ H\ \ H \\ CH_3\ .\ CH(OH)\ .\ C\ .\ C\ .\ C\ .\ CHO \\ \qquad\quad H\ \ HO\ \ HO \end{array}$$

Characteristic is the formation of methyl furfuraldehyde on distillation with acids. Rhamnose forms a phenyl osazone, and by means of the cyanohydrin reaction has been converted into rhamnohexose and rhamnoheptose.

Other methyl pentoses are: chinovose, only known in the glucoside chinovin; fucose, which, as the polymer fucosan, is a component of the cell wall of many seaweeds; and rhodeose, obtained from the glucoside convolvulin, which has been proved to be the optical antipode of fucose. Fucose has the constitution :—

$$\begin{array}{l} \qquad\quad H\ \ H\ \ OH \\ CH_3\ .\ CH(OH)\ .\ C\ .\ C\ .\ C\ .\ CHO \\ \qquad\quad OH\ \ OH\ \ H \end{array}$$

Mention may be made of an altogether abnormal sugar, termed apiose, on account of its presence in the glucoside apiin. This contains a branched chain of carbon atoms, having the formula :—

$$\begin{array}{l} CH_2OH \\ \qquad\quad \diagdown \\ \qquad\quad C(OH)\ .\ CH(OH)\ .\ CHO \\ \qquad\quad \diagup \\ CH_2OH \end{array}$$

The Carbohydrate Alcohols.—Several of the carbohydrate alcohols are widely distributed in the vegetable kingdom; they occur, as a rule, uncombined with other substances.

Erythritol, $C_4H_{10}O_4$, is found in many algæ and mosses; it is optically inactive, and has a sweet taste.

The only naturally occurring pentose alcohol is adonitol, $C_5H_{12}O_5$, corresponding to ribose. Arabitol and xylitol are obtained on reduc-

tion of the corresponding aldoses. The hexose alcohols have been already described (p. 25). *d*-Mannitol and *d*-dulcitol are widely distributed in nature; *d*-sorbitol is more rare, but it can be obtained without difficulty from ripe mountain-ash berries. Two heptose alcohols, $C_7H_{16}O_7$, are known, *e.g.*, perseitol, occurring in *Persea gratissima*, and volemitol, discovered in *Lactarius volemus*, and since identified in the rhizomes of some species of primula. Perseitol is the alcohol corresponding to mannoheptose.

An octitol has been isolated from the mother liquors of the sorbitol preparation from the fruit of some of the *rosaceæ*.

These alcohols are similar in properties to mannitol. Their physical constants are collected in Table VII.:—

TABLE VII.

Alcohol.	Melting-point.	Optical Rotatory Power $[\alpha]_D$.
Erythritol	126°	inactive
Adonitol	102°	inactive
Mannitol	168°	+ 22·5°
Dulcitol	188°	inactive
Sorbitol	110°	+ 12·3°
Perseitol	180°	− 1·3°
Volemitol	154°	+ 1·9°

CHAPTER IV.

THE DISACCHARIDES.

THE disaccharides are carbohydrates containing twelve carbon atoms, and consist of two simple six-carbon atom residues united through an oxygen atom. They are thus analogous to the simple glucosides, and when acted upon by hydrolytic agents—acids or enzymes—they break down with the addition of a molecule of water into their constituent simpler hexoses, which may be either aldoses or ketoses :—

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

One of the constituent hexoses functions in the same manner as glucose does in the methyl glucosides : the aldehydic or ketonic group of the second hexose may remain functional or it may disappear. In the former case the disaccharide reduces cupric salts, forms an osazone, and exhibits mutarotation behaving just as glucose does ; in the latter all these properties are absent. Accordingly the disaccharides are classified under two types.

The following table contains the better-known disaccharides with their component hexoses and optical rotatory power. Some trisaccharides are also included ; also the tetrasaccharide, stachyose :—

TABLE VIII.

DISACCHARIDES.

Sugar.	Components.	Rotatory Power.
	Type 1.—*Aldehyde Group Potentially Functional.*	
Maltose	Glucose-α-glucoside	+ 138°
Isomaltose	Glucose-β-glucoside	?
Gentiobiose	Glucose-β-glucoside	+ 9·6°
Cellobiose	Glucose-β-glucoside	+ 33·7°
Lactose	Glucose-β-galactoside	+ 52·5°
Isolactose	Glucose-galactoside	?
Melibiose	Glucose-galactoside	+ 143°
Turanose	Glucose and fructose	+ 71·8°
	Type 2.—*No Reducing Properties.*	
Sucrose	Glucose and fructose	+ 66·5°
Trehalose	Glucose and glucose	+ 197°

TABLE VIII. (*continued*).

Sugar.	Components.	Rotatory Power.
	TRISACCHARIDES.	
Type 1.		
Mannotriose	Glucose + galactose + galactose	+ 167°
Rhamninose	Glucose + rhamnose + rhamnose	− 41°
Type 2.		
Raffinose	Galactose + glucose + fructose	+ 104°
Gentianose	Glucose + glucose + fructose	+ 33°
Melicitose	Glucose + glucose + fructose	+ 94°
	TETRASACCHARIDE.	
Type 2.		
Stachyose	Fructose + glucose + galactose + galactose	+ 148°

The disaccharides of type 1 form sparingly soluble phenyl osazones, which are difficult to purify, similar to one another, and do not show sharp melting-points as they decompose at the melting-point ; moreover, both melting-point and crystalline form are greatly altered by small quantities of impurities. The hydrazones, even those prepared from asymmetrically disubstituted phenyl hydrazines, are too soluble, as a rule, to be used for their isolation from aqueous solutions.

The difficulty attending research in this group lies in the fact that no really characteristic derivatives of the disaccharides, by means of which they can be isolated and identified with certainty, are known, and partly for this reason but little progress has been made in the direction of their synthesis.

Maltose, lactose and melibiose, which reduce Fehling's solution, form hydrazones and osazones with phenyl hydrazine and combine with hydrogen cyanide, contain, like glucose, an aldehyde group or its equivalent. Since they all show mutarotation, and exist in two modifications, there is no doubt that, like glucose, they possess a closed-ring structure rather than a free aldehyde group. In solution they exist as an equilibrated mixture of dynamic isomerides. Both halves of the molecule thus possess a γ-oxidic structure, one section only retaining the aldehyde group potentially functional.

Interest in the configuration of the disaccharides centres round three main points :—

(1) The nature of the component hexoses.

(2) Whether they represent α- or β-glucosides.

(3) Which hydroxyl group functions in the attachment of the two hexose residues ?

The solution of the first of these problems is a simple matter. The second question has been answered in two ways : firstly, by studying

the behaviour of the sugar towards maltase and emulsin—if hydrolysed by the former it is an α-glucoside, if by the latter a β-glucoside; secondly, by studying the optical behaviour of the glucose immediately produced, on hydrolysing the sugar with an enzyme, towards a drop of alkali—downward mutarotation classes it as α-glucose, upward mutarotation indicates the presence of β-glucose. The third question has not yet been satisfactorily solved; so far it has been only possible to show for maltose and lactose that certain groups are not concerned in the junction.

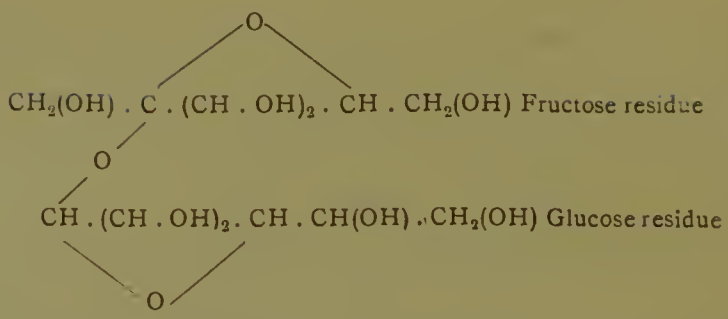
In the following pages the individual disaccharides are briefly dealt with. The problems connected with their hydrolysis and synthesis are deferred to Chapter VI.

Sucrose.—Sucrose or cane sugar, industrially the most important of the sugars, is widely distributed in the vegetable kingdom, where it functions almost entirely as a reserve material. In contrast to most of the sugars, it crystallises exceedingly well: this is almost certainly due to the fact that a mixture of isomerides is not present in solution. It is very soluble in water, and has a much sweeter taste than glucose, but is not so sweet as invert sugar.

Cane sugar does not reduce Fehling's solution or exhibit mutarotation, and it lacks both aldehydic and ketonic properties. Very characteristic is the behaviour towards mineral acids which hydrolyse it to glucose and fructose. Sucrose is dextrorotatory, but, since fructose is more laevorotatory than glucose is dextrorotatory, the products of hydrolysis rotate polarised light in the opposite sense to cane sugar. The process is hence termed inversion, and the product invert sugar. The like change is brought about by an enzyme present in yeasts, moulds, and in many plants, and termed invertase or sucrase. Cane sugar is fermented by yeasts only after previous inversion with the invertase of the yeast. Accordingly it is not fermented by yeasts which do not contain invertase, *e.g.*, *S. octosporus*.

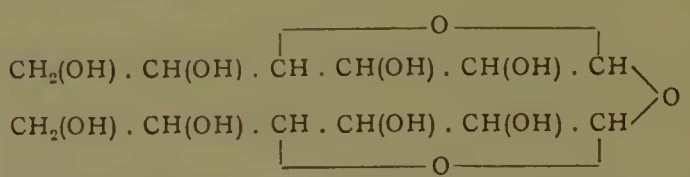
Sucrose forms no compounds with phenyl hydrazine, and is stable towards alkali. It also does not give rise to glucosidic derivatives. It contains eight hydroxyl groups, as evidenced by the formation of an octa-acetate and an octamethyl derivative.

It is not easy to ascribe a constitutional formula to cane sugar which is entirely satisfactory. Fischer's formula, which is a modification of the earlier one of Tollens, pictures it at one and the same time as a glucoside and a fructoside. The glucose and fructose units are joined so as to destroy both aldehyde and ketone groups and give a neutral product:—



The observations of O'Sullivan and Tompson showed that a glucose of high positive rotatory power is at first produced on hydrolysis, *i.e.*, cane sugar is a derivative of *a*-glucose. Yet, inasmuch as it is not attacked by maltase, which acts on all simple *a*-glucosides, it cannot well belong to their class. Moreover, since Pottevin has shown that the simple methyl fructoside is not hydrolysed by the enzymes which attack sucrose, it must be supposed that cane sugar is not a simple fructoside. The extraordinary instability of sucrose in presence of acids also differs markedly from the behaviour of the simple glucosides. Invertase is remarkably active in hydrolysing sucrose. Its action seems to be controlled and inhibited by both glucose and fructose, and apparently the enzyme is so constituted that it can adapt itself to both sections of the biose. The question is further discussed in Chapter VI.

Trehalose.—Trehalose, which occurs widely distributed in fungi, is composed of two glucose molecules fused together, so that both aldehydic groups have disappeared :—



This structure is indicated by the fact that it does not reduce Fehling's solution, nor form a phenyl osazone nor exhibit mutarotation. It is not affected by the enzymes, maltase, invertase, emulsin or diastase, but is hydrolysed by a special enzyme named trehalase, which is contained in certain fungi and in many species of yeast. Trehalase is conveniently obtained from *aspergillus niger*. According to Winterstein trehalose is only hydrolysed by acids with considerable difficulty, and contrasts markedly in this respect with sucrose.

Apparently trehalose replaces sucrose in those plants (fungi) which contain no chlorophyll and do not manufacture starch. The quantity of trehalose is a maximum just before the formation of spores. When

the fungi are picked the trehalose is rapidly converted into mannitol, being hydrolysed by its enzyme to glucose, and this in some way then reduced. To obtain it the fungi must be extracted with boiling solvents, so as to kill the enzyme, within two or three hours after gathering.

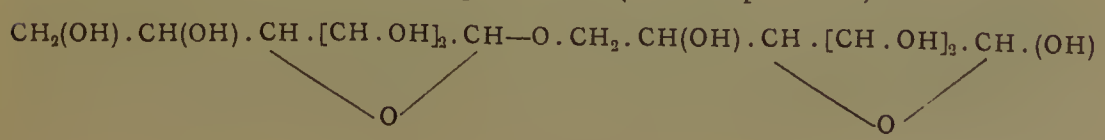
Maltose.—A sugar was first isolated from the products of hydrolysis of starch by De Saussure in 1819, but it was not until 1847 that this new sugar was further examined by Dubrunfaut and named maltose. This discovery seems to have lapsed into comparative oblivion until the sugar was rediscovered by O'Sullivan in 1872. Maltose is prepared by the action of diastase on starch, the only other product of the change being dextrin. It crystallises in minute needles, has a high dextrorotatory power and exhibits upward mutarotation, *i.e.*, the rotatory power when the disaccharide is first dissolved is smaller than the equilibrium value.

Maltose reduces Fehling's solution, forms a phenyl osazone, and shows many other of the properties of glucose.

When hydrolysed by acids two molecules of glucose are formed. It is very much more resistant to acid hydrolysis than cane sugar.

The enzymes diastase, invertase, lactase and emulsin are without action, maltase alone of all the known enzymes being able to effect hydrolysis. Maltose is fermented only by those yeasts which contain maltase, and then not until inversion has been brought about by the enzyme. In view of the behaviour of maltose towards maltase, it is considered to be a glucose-*α*-glucoside, since it is only *α*-glucosides which are hydrolysed by maltase; and in confirmation of this view *α*-glucose has been proved to be formed initially on hydrolysis.

Maltose yields, on oxidation with bromine, an acid containing the same number of carbon atoms, which is termed maltobionic acid; this is hydrolysed to glucose and gluconic acid by mineral acids. Maltose combines with hydrogen cyanide, forming a compound which, on hydrolysis, gives maltose carboxylic acid, and is hydrolysed by mineral acids to glucose and glucoheptonic acid. Maltose must contain eight hydroxyl groups, as it gives an octa-acetyl derivative when acetylated. The behaviour of maltose is in accord with the constitutional formulæ below. As already stated, it is not known which carbon atom is concerned in the attachment of the two sugar residues. Provisionally, the terminal carbon atom is so represented (see Chapter VI.) :—



Maltose forms a glucoside analogous to methyl glucoside, but the direct condensation with methyl alcohol in presence of acid is not possible, as the disaccharide becomes hydrolysed during the operation. β-Methyl maltoside has been prepared from acetochloro maltose, obtained by the action of hydrogen chloride on maltose octa-acetate. Acetochloro maltose interacts with methyl alcohol in presence of silver carbonate, forming hepta-acetyl methyl maltoside, which is converted into methyl maltoside on hydrolysis with baryta. The behaviour of this maltoside towards enzymes is interesting. Maltase hydrolyses it at the α-junction, forming glucose and β-methyl glucoside; emulsin attacks only the β-junction, forming maltose and methyl alcohol. The maltoside is accordingly β-methyl glucose-α-glucoside.

Isomaltose.—Isomaltose is the name given by Fischer to the disaccharide obtained by him by the condensing action of strong acids on glucose. It was characterised only by means of the phenyl osazone and the fact that it is not fermented by yeast. Products similar to isomaltose have been repeatedly described as obtained in the hydrolysis of starch, but, failing any characteristic derivative, definite proof of its presence in such cases is lacking. Isomaltose is probably identical with the disaccharide obtained by Croft Hill by the synthetic action of maltase on glucose (see Chapter VI.) which he has termed revertose. E. F. Armstrong has shown that isomaltose is hydrolysed by emulsin, but not by invertase or maltase, and considers the isomaltose obtained by means of acids or enzymes to be the same in each case. The behaviour towards emulsin and maltase suggests that it is probably glucose β-glucoside.

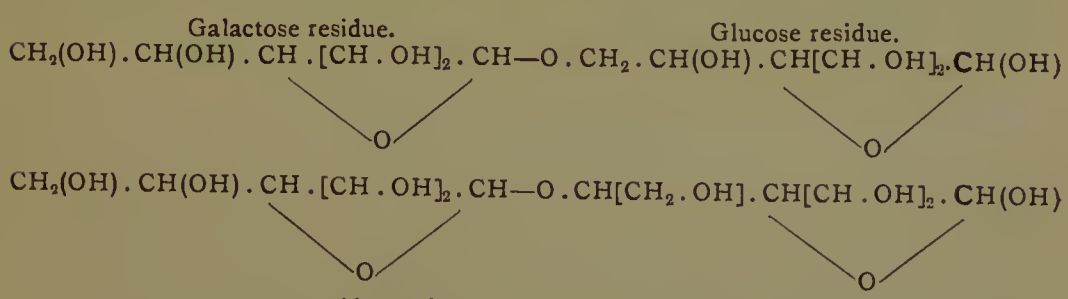
Gentiobiose.—Gentiobiose is closely allied to maltose and isomaltose. It is found in the form of a trisaccharide termed gentianose present in the roots of various species of gentians. When partially hydrolysed either by means of invertase or dilute acids, this yields fructose and gentiobiose. Gentiobiose forms a phenyl osazone, m.-p. 142°, shows mutarotation, and is hydrolysed by emulsin: it is supposed to be a β-glucoside.

Cellobiose.—Cellulose (filter paper), when acetylated under suitable conditions (Skraup), forms an octa-acetyl disaccharide, among other products, from which the corresponding sugar termed cellobiose is obtained on hydrolysis with alkali. The cellobiose reduces Fehling's solution, and forms a phenyl osazone and osone in the same way as maltose. Fischer has shown that it is hydrolysed by emulsin, and it is therefore presumably a β-glucoside. He points out, however, that, inasmuch as emulsin is known to be a mixture of enzymes, it is not

certain that the same enzyme which hydrolyses β-methyl glucoside also resolves isomaltose, gentiobiose and cellobiose (see also p. 78).

Lactose.—Lactose or milk sugar, discovered in 1615 by Fabriccio Bartoletti in Bologna, occurs in the milk of all animals, but has not been encountered in the vegetable kingdom. It is manufactured by evaporation of whey, purified by recrystallisation, and obtained in the form of a white crystalline powder. Mineral acids hydrolyse it to glucose and galactose; it exhibits mutarotation, reduces Fehling's solution, and forms a phenyl osazone soluble in boiling water. Like glucose, it gives rise to two series of isomeric derivatives, *e.g.*, octa-acetates, acetochloro lactoses and methyl lactosides. Three isomeric modifications of the sugar itself have been described corresponding to the α- and β-isomerides and their equilibrated mixture. It is a glucose galactoside, since, on oxidation with bromine, lactobionic acid is formed, and this when hydrolysed by mineral acids gives gluconic acid and galactose, proving that the potential aldehyde group is in the glucose part of the molecule.

Adopting Fischer's glucoside formula for lactose, it is a question, as previously indicated, whether the primary alcohol group or the δ-secondary alcohol group of the glucose molecule take part in the union with the galactose. The possibility of either the α- or γ-secondary alcohol groups being concerned is excluded by the facts that lactose forms a phenyl osazone, exhibits mutarotation, and gives rise to derivatives having a γ-oxide structure. The β-secondary alcohol group can also be excluded from consideration, as Ruff and Ollendorf have obtained, on oxidising the calcium salt of lactobionic acid by Fenton's method, a galactoarabinose sugar which forms a phenyl osazone in which this β-alcohol group is involved. It must therefore be uncombined in the parent lactose. It is impossible at present to go any further in deciding in favour of either of the remaining two formulæ for lactose (see pp. 65, 66).



Alternative Formulæ for Lactose.

The isomeric α- and β-forms of milk sugar originally described by Tanret and investigated more recently by Hudson, differ only with

respect to the relative positions of the hydrogen and hydroxyl radicles attached to the carbon atom printed in Clarendon type in the glucose half of the molecule. Tanret's γ-lactose is an equilibrated mixture. α-Lactose is properly α-glucose-β-galactoside, whereas β-lactose is β-glucose-β-galactoside.

Galactoarabinose is of interest as the only example of a synthetical mixed disaccharide containing both hexose and pentose sugars. It is therefore akin to the natural sugar rhamninose. The formation of galactoarabinose affords additional proof that lactose is a galactoside.

Lactose is hydrolysed by a specific enzyme lactase found in a few yeasts (or, more correctly, torulæ), in some kefir preparations, and in the enzyme (crude emulsin) contained in an aqueous extract of almonds. It is believed that kefir lactase and almond lactase are not identical. Lactose is not hydrolysed by maltase, invertase, diastase, nor by any of the enzymes of dried brewers' yeast. Only those yeasts (torulæ) which contain lactase are capable of fermenting milk sugar. Lactose is particularly prone to undergo lactic and butyric acid fermentations.

Isolactose is the name given to a disaccharide obtained by Fischer and Armstrong by the synthetical action of the enzyme kefir lactase on a concentrated solution of equal parts of glucose and galactose, and isolated in the form of the phenyl osazone. It has not been further studied.

Melibiose.—Melibiose, together with fructose, is obtained from the trisaccharide raffinose by hydrolysis with dilute acids or certain yeasts (Scheibler and Mittelmeier). It crystallises with difficulty and it is advisable to remove the fructose from the products of hydrolysis of raffinose by fermentation with a top yeast before attempting to isolate it. On hydrolysis with strong acids melibiose yields glucose and galactose. On reduction with sodium amalgam an alcohol, melibiitol is formed. This, when hydrolysed, is converted into mannitol and galactose. Melibiose is thus a galactoside of glucose, *i.e.*, very closely related to milk sugar.

It exhibits mutarotation, forms a phenyl osazone and an osone which latter decomposes to galactose and glucosone.

Melibiose is slowly hydrolysed by emulsin, more rapidly by an enzyme contained in bottom fermentation but not in top fermentation yeasts: this enzyme is appropriately termed melibiase. Melibiose is not attacked by maltase, invertase or lactase. It affords a chemical means of distinguishing between top and bottom fermentation yeasts. It is apparently less easily hydrolysed by acids than is milk sugar.

The difference between melibiose and milk sugar appears to depend

upon which hydroxyl of the glucose molecule is united to the galactoside. (See types A and B, p. 65.) Since both disaccharides are attacked by emulsin they may provisionally both be considered as β-galactosides.

Added interest attaches to melibiose in view of its being the first natural disaccharide obtained synthetically (Fischer and Armstrong, see p. 72).

Melibiosone, which can be prepared from the osazone by heating with benzaldehyde, is hydrolysed by emulsin or by melibiase to galactose and glucosone.

Turanose.—Turanose was discovered by Alechin in 1890 as a product, together with fructose, of the partial hydrolysis of a trisaccharide melecitose with weak acids. He stated that it yielded two molecules of glucose on further hydrolysis but Tanret subsequently showed that an equimolecular mixture of glucose and fructose is produced. It is thus an isomeride of sucrose but differs from this in containing a free aldehydic group since it forms a phenyl osazone and reduces Fehling's solution. It does not exhibit mutarotation. It is not at present known whether it is to be regarded as a fructoside or a glucoside. Invertase, maltase, emulsin and diastase are without action.

Trisaccharides, $C_{18}H_{32}O_{16}$.

Raffinose.—The best-known trisaccharide is raffinose which is often found in considerable amount in the sugar beet, and is present in other plants. Strong mineral acids hydrolyse it completely to fructose, glucose and galactose in equal proportions. Dilute acids form melibiose and fructose. The action of enzymes on raffinose is more specialised; invertase converts it into fructose and melibiose. Emulsin, however, hydrolyses it to sucrose and galactose. Bottom yeasts which contain both melibiase and invertase are able to ferment it completely.

Raffinose has no reducing action and behaves chemically as cane sugar. The constitutional formula may be written—

$$\underbrace{C_6H_{11}O_5}_{\text{Fructose}}—O—\underbrace{C_6H_{10}O_4}_{\text{Glucose}}—O—\underbrace{C_6H_{11}O_5}_{\text{Galactose}}$$

$$\underbrace{\qquad\qquad}_{\text{Sucrose.}} \qquad \underbrace{\qquad\qquad}_{\text{Melibiose.}}$$

Gentianose.—Gentianose is a non-reducing sugar, and it yields fructose and gentiobiose when hydrolysed by invertase. It is not attacked by maltase nor as a rule by emulsin but according to Bourquelot some emulsin preparations hydrolyse it slowly forming glucose and sucrose.

Rhamninose.—Rhamninose, the trisaccharide derived from the glucoside xanthorhamnin by the action of the enzyme rhamninase, is composed of galactose and rhamnose; it reduces Fehling's solution and forms an acid, when oxidised with bromine, which on hydrolysis by mineral acids is broken down to two molecules of rhamnose and one of galactose. This indicates that the two rhamnose residues are united in the trisaccharide but the separation of the dirhamnose sugar has not been effected. The enzymes, invertase, maltase, emulsin and diastase are quite without action.

CHAPTER V.

THE RELATION BETWEEN CONFIGURATION[1] AND BIOCHEMICAL PROPERTIES.

PERHAPS the most important, and at the same time the most interesting, chapter in the chemistry of the sugars is that dealing with the alteration in properties brought about by small changes in the stereo-chemical configuration of the carbohydrate molecule. Although the molecular weight and the gross structure of the molecule remain the same, the very slightest modification in the space arrangement of the groups attached to the chain of carbon atoms is sufficient to affect the biochemical behaviour in the most profound manner. How exactly structure is to be correlated with biological behaviour, and how little variation is possible, will be seen from the following examples.

It has long been known that the optical antipodes of a substance containing an asymmetric carbon atom behave very differently towards biological agents, such as yeasts, moulds, enzymes or bacteria. The celebrated researches of Pasteur showed, for example, that the green mould *penicillium glaucum*, when allowed to grow in solutions of racemic acid, assimilated only *d*-tartaric acid, and left *l*-tartaric acid untouched. It was supposed at the time that the mould was unable to attack the *l*-tartaric acid; recent investigations suggest, however, that the mould ultimately destroys both antipodes, but attacks one at a very much greater rate than the other, and probably in a different manner.

From a given racemic substance it is possible to obtain sometimes the one and sometimes the other antipode by utilising appropriate organisms. For example, an excess of *d*-mandelic acid is obtained from *dl*-mandelic acid on treatment with *penicillium glaucum*, whereas when *saccharomyces ellipsoideus* is used an excess of *l*-mandelic acid is obtained.

[1] By the term configuration is understood the positions of the hydroxyl groups relative to the skeleton chain of carbon atoms. Change involves transference from the right to left side of the chain as figured on the plane of the paper or *vice versâ* from left to right.

Fermentation.—Yeasts only ferment one, the dextro, isomeride of glucose converting it into carbon dioxide and alcohol, and accordingly when yeasts are allowed to act on racemic glucose the laevoglucose remains unattacked. The same applies to the other fermentable hexoses; in all cases only the dextro isomeride is attacked.

The investigation of the behaviour of all the known hexoses, either found in nature or prepared in the laboratory, towards yeasts has shown that only four are fermented, *viz.*, the *d*-forms of glucose, mannose, galactose and fructose, all of which are natural products.

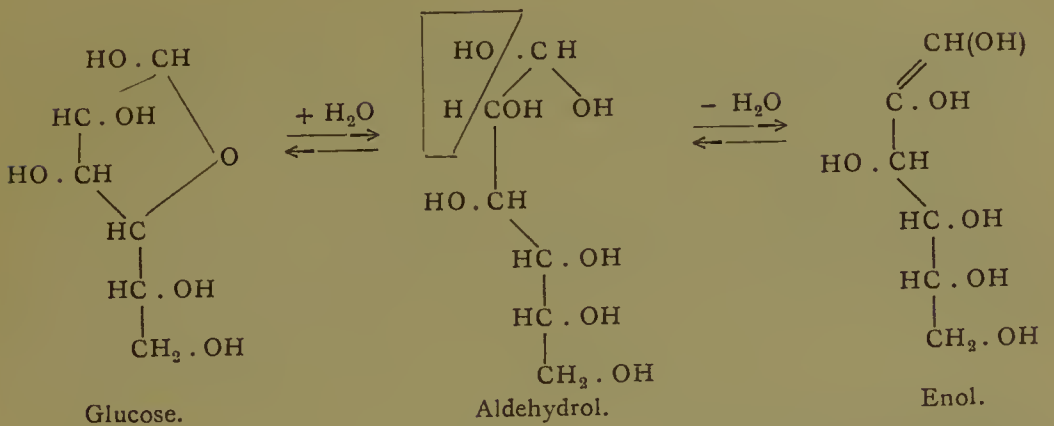
When the behaviour of different species of yeasts towards these natural hexoses is studied, it is found without a single exception that any species of yeast which ferments any one of the three hexoses—glucose, mannose and fructose—likewise ferments all three of them, and with approximately the same readiness. The study of the kinetics of the three fermentation reactions confirms their similarity, and they have the same temperature coefficient (Slator). Everything, in fact, points to the mechanism involved in the fermentation of glucose, mannose or fructose being the same in each instance.

It has already been pointed out that the three hexoses in question are closely related in structure, so closely indeed as to be converted under the influence of alkalis into one another. An enolic form common to all three hexoses has been assumed to act as an intermediate substance in the transformation. The relationship will become clear when the formulæ of these carbohydrates are consulted:—

CHO	CHO	CH.OH	CH_2.OH
HĊOH	HOĊH	ĊOH	ĊO
HOCH	HOCH	HOCH	HOCH
HCOH	HCOH	HCOH	HCOH
HCOH	HCOH	HCOH	HCOH
CH_2OH	CH_2OH	CH_2OH	CH_2OH
Glucose.	Mannose.	Common enolic form.	Fructose.

It is clearer here to use the older open-chain formulæ, but the reader is advised to study these formulæ in the solid model in order to understand fully the stereoisomerism of these compounds. Representations on a plane surface easily lead to confusion.

On the basis of the closed-ring formula for glucose, enolisation involves in the first place rupture of the pentaphane ring and formation of the aldehydrol; secondly, water is eliminated between two contiguous carbon atoms to give the enol. Comparing the scheme opposite with that on p. 4, for the conversion of the aldehydrol into glucose, the difference is at once apparent:—



Glucose. Aldehydrol. Enol.

According to the alternative formula the aldehyde forms aldehydrol and this enol. The change is a reversible one.

The process of fermentation of a sugar is regarded as a series of consecutive reactions each involving simplification of the sugar molecule till it breaks down into carbon dioxide and ethyl alcohol, compounds containing only one and two carbon atoms. Measurements of the rate of fermentation can be made by determining the rate of formation of either of these products—for example, the amount of CO_2 formed after various intervals of time—but such measurements only apply to the slowest of these reactions. Similarly the quantitative effect produced by an increase of temperature in quickening the rate of fermentation in reality applies to the slowest reaction of the series.

It has been suggested that the first process in fermentation is the conversion of the sugar into the enolic form by means of an enzyme contained in the yeast. The three fermentable hexoses yield the same enolic form, but possibly it is formed at different rates according to the sugar; and whether one and the same enzyme is operative in each case it is impossible to say. The subsequent simplification of the molecule is the same for each of the three hexoses, an hypothesis which is quite in agreement with the experimental observations. This simplification is also due to an enzyme perhaps the same as that which brought about enolisation. The breakdown of the molecule will thus commence at the double linkage between the two terminal carbon atoms.

Further support of this view of the fermentation process is afforded by the fact that substances so closely related to glucose as the methyl glucosides, glucosone, gluconic acid and ethyl gluconate are, without exception, unfermentable: in all these only the groups attached to the terminal carbon atom differ from those of glucose. Enolisation in them, however, is impossible, and, although the greater part of the molecule is not altered, no action takes place.

The behaviour of galactose is altogether different. It is fermented with much greater difficulty than glucose. Very many yeasts are quite without action on galactose. The temperature coefficient of the fermentation of galactose is different from the value found in the case of glucose. These facts suggest that galactose is fermented by a different mechanism, that a different enzyme is concerned perhaps in causing enolisation, which is less widely distributed in yeasts. None the less the two phenomena must be very closely allied. No yeast is known capable of fermenting galactose but not fermenting glucose.

The change in configuration in passing from glucose to galactose, though not sufficient to prevent fermentation altogether, causes the compound to be far more resistant to attack. It is not surprising, therefore, that any further change in configuration is sufficient to make the new hexose no longer fermentable.

This is illustrated by the behaviour of galactose and its isomerides, talose and tagatose, which have an enolic form common to all three hexoses :—

CHO	CHO	CH_2OH	CHOH
HĊOH	HOĊH	ĊO	ĊOH
HOCH	HOCH	HOCH	HOCH
HOCH	HOCH	HOCH	HOCH
HCOH	HCOH	HCOH	HCOH
CH_2OH	CH_2OH	CH_2OH	CH_2OH
Galactose.	Talose.	Tagatose.	Enolic form.

Neither talose nor tagatose are fermented by any yeast whose action towards them has at present been investigated. Yet in talose the position of the two upper hydroxyl groups is the same as that in mannose, and the lower three hydroxyls occupy the same positions as they do in galactose. Obviously, for it to be fermentable, the configuration of the hexose has to be correct as a whole, the fact that single hydroxyl groups occupy the same positions as they do in fermentable hexoses being of no moment.

Presumably yeasts contain no enzymes compatible with talose or tagatose, and able to convert them into the enolic form.

The facts described can only be explained on the assumption that there is the very closest relationship between the configuration of a fermentable hexose and the enzymes which cause fermentation. This hypothesis receives confirmation which is little short of absolute when the behaviour of the sugars other than the hexoses is considered. No pentose, either natural or synthetical, is fermentable by yeast. None of the synthetic tetrose, heptose or octose carbohydrates are fermentable.

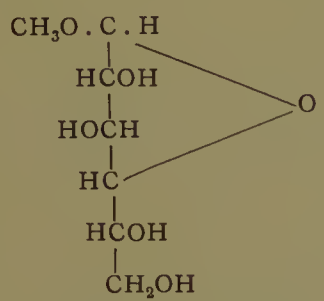
The only fermentable sugars, other than the four hexoses, are a

nonose prepared by the cyanohydrin reaction from mannose and a ketotriose, *dioxyacetone*. The fermentability of glycerose—a mixture of glyceric aldehyde and dioxyacetone—has long been a matter of controversy; Bertrand has, however, shown that pure dioxyacetone is fermented by very active yeasts.

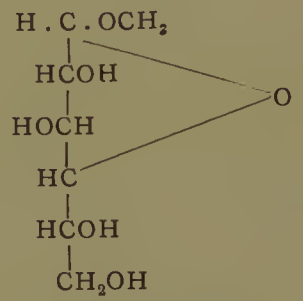
It is obvious how intimately the property of undergoing fermentation is connected with the configuration of the sugar molecule. Lengthening or shortening the chain of carbons is sufficient to place the sugar molecule out of harmony with the yeast enzymes, and thus prevent its destruction by fermentation. The fact that triose, hexose and nonose sugars are fermentable has led to the suggestion that the fermentable carbohydrates must contain a multiple of three carbon atoms.

Glucoside Hydrolysis.—The formation of stereoisomeric *α*- and *β*-methyl glucosides by the interaction of glucose and methyl alcohol in presence of hydrogen chloride has already been discussed and their constitutional formula established. These isomeric glucosides, though so alike in structure, behave very differently towards enzymes.

α-Methyl glucoside is hydrolysed by the *maltase* (*α*-glucase[1]) of yeast, *β*-methyl glucoside by *emulsin* (*β*-glucase) which is widely distributed in plants. Emulsin is quite without action on the *α*-glucoside; maltase has no effect on the *β*-glucoside.



α-Methyl glucoside
hydrolysed by Maltase.



β-Methyl glucoside
hydrolysed by Emulsin.

Other alkyl derivatives of glucose behave in a similar manner. It may be stated as a general rule that *β*-glucosides are hydrolysed by emulsin alone, *α*-glucosides are only attacked by maltase. Accordingly compounds hydrolysed by emulsin are considered to be *β*-glucosides. The corresponding derivatives of *l*-glucose are not in the slightest affected by either enzyme. *α*- and *β*-methyl-*l*-glucosides represent the

[1] *Nomenclature of Enzymes.*—The name of an enzyme is usually derived from that of the sugar which it hydrolyses by substituting the suffix *-ase* for *-ose*. Thus maltase hydrolyses maltose, lactase hydrolyses lactose. The enzyme which attacks glucosides may be termed *glucase* and is an *α*-glucase or *β*-glucase accordingly as it hydrolyses the *α*- or *β*-glucoside.

mirror images of the methyl-*d*-glucosides and their behaviour is parallel to that of *l*-glucose towards living yeast.

The glucosidic derivatives of mannose, *viz.*, methyl-*d* and *l*-mannoside are also quite stable in presence of maltase or emulsin. Hence the change in position of a single hydroxyl (here that attached to the *a*-carbon atom) is sufficient to render the mannoside out of harmony with these enzymes; but, as has just been seen, the change in configuration is not sufficient to make mannose unfermentable by yeast.

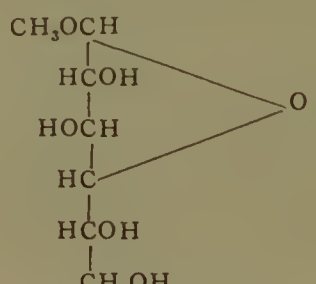
a-Methyl-*d*-galactoside is likewise not hydrolysed by maltase or emulsin.

β-Methyl-*d*-galactoside is hydrolysed by the crude emulsin preparation obtained from almonds, but subsequent investigation has shown that this preparation contains a mixture of enzymes and that the hydrolysis of the *β*-galactoside is due to a lactase (*β*-galactase) and not to the same enzyme which attacks *β*-methyl glucoside. This behaviour shows that the alteration in the position of the hydroxyl attached to the *γ*-carbon atom in the glucoside molecule renders the galactosides out of harmony with maltase and emulsin. Any other alteration involving departure from the configuration of the glucose molecule or in the length of the chain of carbon atoms has the same effect on the behaviour towards enzymes.

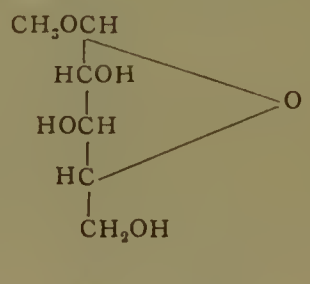
None of the known glucosides[1] of the pentoses, methyl pentoses, heptoses or other hexoses are hydrolysed by maltase or emulsin.

This behaviour can only mean that the hydrolysing power of these two enzymes bears the very closest relationship to the configuration of the dextro glucose molecule.

Fischer has drawn particular attention to the behaviour of the *a*- and *β*-methyl-*l*-xylosides. These practically correspond to the corresponding glucosides with one asymmetric carbon atom removed:—



a-Methyl-*d*-glucoside.



a-Methyl-*d*-xyloside.

Both xylosides are unaffected by either maltase or emulsin. In

[1] The term glucoside is used generally for the corresponding derivatives of all the sugars and not restricted to the derivatives of glucose.

this instance, although the major part of the molecule is identically the same in each glucoside, the shortening of the chain is sufficient to destroy the close harmony with the enzyme. The glucosides investigated by Fischer are summarised in the following table in which + indicates hydrolysis, o denotes no action :—

TABLE IX.

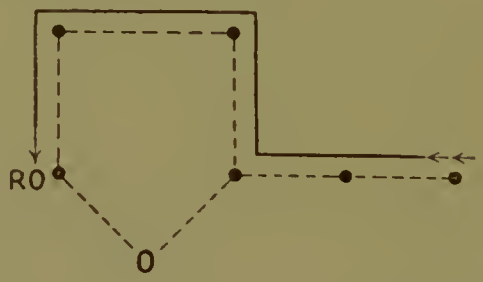
Glucoside.	Maltase (α-Glucase).	Emulsin (β-Glucase).
α-Methyl-*d*-Glucoside . . .	+	o
β-Methyl-*d*-Glucoside . . .	o	+
α-Methyl-*l*-Glucoside . . .	o	o
β-Methyl-*l*-Glucoside . . .	o	o
α-Ethyl-*d*-Glucoside . . .	+	o
β-Ethyl-*d*-Glucoside . . .	o	+
β-Phenol-*d*-Glucoside . . .	o	+
α-Methyl-*d*-Galactoside . .	o	o
β-Methyl-*d*-Galactoside . .	o	o
Methyl-*d*-Mannoside . .	o	o
Methyl-*l*-Mannoside . .	o	o
α-Methyl-*l*-Xyloside . . .	o	o
β-Methyl-*l*-Xyloside . . .	o	o
Methyl-*l*-Arabinoside . .	o	o
Methyl rhamnoside . . .	o	o
Methyl glucoheptoside . .	o	o

The investigation of the rate of hydrolysis of maltose—an α-glucoside—by maltase has shown that change takes place more slowly in the presence of glucose, indicating that this sugar has a definite retarding influence on the enzyme. Other sugars, *e.g.*, mannose, fructose, galactose, arabinose, xylose are quite without influence on the rate of change proving that the action of glucose is due not to any concentrating effect but to the specific influence exerted by its configuration. The fact that β-methyl glucoside also acts to retard the hydrolysis of the α-glucoside (maltose) affords the strongest confirmatory evidence of this specific hindrance. Part of the enzyme must combine with the glucose and so be withdrawn from action. Maltase can apparently combine with β-methyl glucoside though quite unable to hydrolyse it.

In an analogous manner the hydrolysis of β-methyl glucoside by emulsin is controlled only by glucose and α-methyl glucoside, and by no other carbohydrate.

These illustrations, selected from a number of carefully worked out cases, suffice to show the very intimate relation which exists between enzyme and the substance it acts upon. This can only be explained by supposing some form of combination between the two. The

enzyme, moreover, must fit the glucoside at every point along the chain of carbon atoms, thus :—



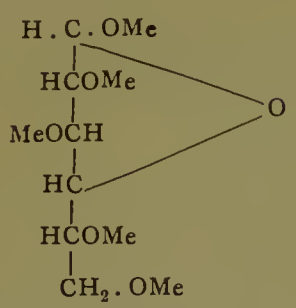
The combination may perhaps be compared to the way in which the successive fingers of a glove fit on to a right hand: if the position of any finger be altered it is impossible to fit the glove; further, the glove will not fit on the left hand. Fischer's original simile compared the relationship of enzyme to hydrolyte to that existing between a key and the lock for which it is made, the shape of the key enabling it only to unfasten the particular lock to the arrangement of whose wards it corresponds.

The enzymes themselves, if this hypothesis be accepted, must be closely related in configuration to the substances which they hydrolyse. From this point of view the presence of a carbohydrate in the molecule of invertase and some other enzymes is at least significant (see Monograph by Bayliss, p. 19). Salkowski states, however, that the carbohydrate present in the yeast gum is precipitated with the enzyme, but that it is not a component of the purified enzyme.

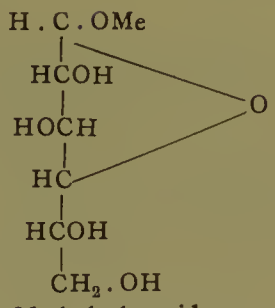
It is perhaps necessary to emphasise that the actual hydrolysis of the carbohydrate is due to the action of the water molecules. The enzymes may be conceived perhaps as acting as a vice in presenting in the appropriate manner the water molecule to the centre to be hydrolysed.

Attachment of enzyme to hydrolyte takes place no doubt through the oxygen atoms of the hydroxyl groups. In these the oxygen atom possesses residual affinity, that is, is not fully saturated, and it is therefore able to combine with appropriate elements of the molecule of the enzyme.

The fact that tetramethyl-β-methyl glucoside like β-methyl glucoside itself is hydrolysed by emulsin is in full agreement with this view :—



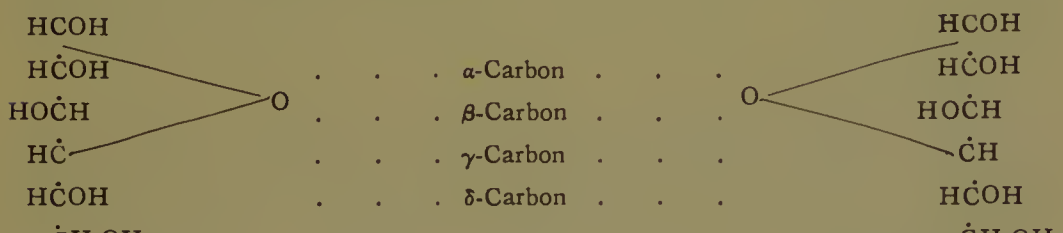
Tetramethyl-β-methyl glucoside.



β-Methyl glucoside.

Although in this compound the hydrogen in the hydroxyl groups of glucose has been replaced by methyl, this change is not sufficient either to destroy the residual affinity of the oxygen atoms or to mask them from the influence of the enzyme.

Conversion of Galactose into Glucose.—When the closed-ring formulæ of the two hexoses, glucose and galactose, are considered side by side, it will be obvious that the difference between them is confined to the relative positions of the groups attached to the 4th or γ-carbon atom, *i.e.*, the oxygen atom of the pentaphane ring is attached to different sides of the molecule :—



Glucose. Galactose.

The direct conversion of one sugar into the other involves the rupture of the ring at this point and its closure again in the opposite sense. The whole behaviour of glucose shows, however, that the pentaphane ring ruptures preferentially at the attachment of the oxygen to the α-carbon atom. The conversion of glucose into galactose has been only indirectly effected by chemical means, but there is little doubt that it takes place in the organism, as it is only on this supposition that the formation of the galactoside, milk sugar, in large quantities in mammals during lactation can be accounted for.

Under normal conditions the blood transports glucose to the mammary glands, where, in the regular course of lactation, it is converted into the disaccharide, milk sugar, and excreted in the milk. Removal of the mammary gland results in an accumulation of glucose in the blood, from which it passes to the urine. Galactose is not found in the urine. Injection of glucose causes lactosuria when the mammary glands are in full activity, but produces glucosuria when the

glands are less active. Nothing is known as to the mechanism by which the mammary glands are able to transform glucose into lactose, but it is undoubtedly effected by means of enzymes.

The enzyme lactase which hydrolyses β-methyl galactoside, other β-alkyl galactosides and milk sugar, is a specific enzyme for β-galactosides, just as emulsin has been shown to be the specific enzyme for β-glucosides. Lactase has its action controlled only by galactose and by no other sugar, and it is incapable of hydrolysing glucosides. No enzyme is at present known which can hydrolyse α-methyl galactoside; on the other hand, no compound of α-galactose is known in nature.

Oxidation.—The influence of configuration has been also studied in the case of the behaviour of carbohydrates towards oxidising bacteria. The *bacterium xylinum* (Adrian Brown), or sorbose bacterium, as it has been termed by Bertrand, oxidises aldoses to the corresponding monobasic acids, and converts the alcohols into ketones, *e.g.*, gluconic acid is formed from glucose; galactonic acid from galactose; xylose and arabinose yield xylonic and arabonic acids. In all these cases the – CHO group is oxidised to – CO_2H by the agency of the bacterium.

In the case of alcohols the sorbose bacteria oxidise – CH(OH) – to – CO –. Thus mannitol forms fructose; sorbitol yields sorbose; erythritol, arabitol and perseitol are oxidised to the corresponding ketones, and glycerol produces dihydroxyacetone. The bacterium has no action, however, on glycol, dulcitol or xylitol.

An examination of the formula of these alcohols shows that the CH(OH) group oxidised to – CO is next to a $CH_2(OH)$ group; further, for action to take place, the hydroxyl group must not be adjacent to a hydrogen atom on the same side of the configuration formula; in other words, the compound must contain the grouping:—

$$\begin{array}{l} \quad H\ \ H \\ CH_2(OH)\ .\ C\ .\ C\ - \\ \quad OH\ OH \end{array}$$

Consideration of the configuration formulæ of mannitol and dulcitol will help to make this clear:—

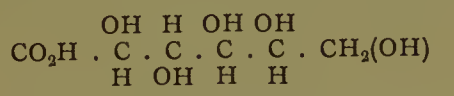
$$\begin{array}{l} \quad H\ OH\ H\ \ H \\ CH_2(OH)\ .\ C\ .\ C\ .\ C\ .\ C\ .\ CH_2(OH) \\ \quad OH\ \ H\ OH\ OH \end{array}$$

Mannitol—converted into Fructose.

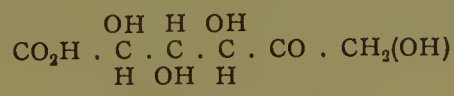
$$\begin{array}{l} \quad H\ OH\ OH\ \ H \\ CH_2(OH)\ .\ C\ .\ C\ .\ C\ .\ C\ .\ CH_2(OH) \\ \quad OH\ \ H\ \ H\ OH \end{array}$$

Dulcitol—not attacked.

Gluconic acid contains the sensitive grouping. Accordingly, it is further oxidised by the bacterium to a keto gluconic acid :—



Gluconic acid.



Keto gluconic acid.

In contrast with the sucroclastic enzymes, which are apparently in harmony with the sugar molecule as a whole, these oxidising bacteria seem adapted to a section only of the molecule. Their action is none the less absolutely dependent on the presence of the requisite configuration in the molecule.

Many bacteria act upon mannitol which are without action on dulcitol. Harden found this to be true for *bacillus coli communis*, which is of interest also since it produces twice as much alcohol from mannitol as from glucose. This difference is ascribed to the presence of the group $CH_2(OH) . CH(OH)$—which is contained once only in glucose but twice in mannitol.

CHAPTER VI.

HYDROLYSIS AND SYNTHESIS.

Hydrolysis of Disaccharides. — Disaccharides are hydrolysed to monosaccharides by mineral and organic acids in accordance with the equation—

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6.$$

Any acid will act on each sugar, though the intensity of the action differs more or less according to the acid or the disaccharide.

The disaccharides are also hydrolysed by enzymes. The action of enzymes is essentially selective: each particular sugar is hydrolysed only by its appropriate enzyme and by no other. There is thus a sharp distinction between the two classes of hydrolysing agents.

Great historical interest attaches to the phenomenon of the hydrolysis of cane sugar by acids as it was one of the first chemical changes of which the course was followed by physical methods.[1] The change in sign of the optical rotatory power on inversion was first announced by Biot in 1836. A few years later Wilhelmy (1850) showed that the amount of sugar changed in any given moment is a constant percentage of the amount of unchanged sugar present. This is known as Wilhelmy's law, and put into mathematical form it is expressed by the equation :—

$$\frac{dx}{dt} = K(a - x)$$

$$\text{or } K = \frac{1}{t}\log_e \frac{a}{a - x}$$

where $\begin{cases} a = \text{initial amount of sugar.} \\ x = \text{amount already inverted.} \\ t = \text{time which has elapsed since the reaction started.} \end{cases}$

This law has been carefully verified experimentally: the above expression is the simplest type of mass action equation. The velocity constant K represents the rate at which the sugar is inverted.

Cane sugar is hydrolysed at very different rates by different acids. If the acids are classified in order according to their power of hydrolysing sucrose they will be found to be also arranged according to their

[1] It is outside the limits of this monograph to do more than indicate the salient features of hydrolysis. A most valuable and complete summary of the literature bearing on the subject with a bibliography complete up to 1906 is contained in a report presented by R. J. Caldwell to the British Association at York, 1906.

electrical conductivity and power of hydrolysing methyl acetate. This fact was first recognised by Ostwald in 1884. Other disaccharides and the glucosides are also hydrolysed by acids in accordance with Wilhelmy's law, but hydrolysis takes place far more slowly than in the case of cane sugar. Indeed, whereas cane sugar is rapidly hydrolysed by normal sulphuric acid at 20°, milk sugar requires prolonged heating at 80° to effect the same proportion of change. Armstrong and Caldwell give the relative ease with which hydrolysis takes place as milk sugar 1, maltose 1·27, cane sugar 1240. Other figures relating to the glucosides are given in Table X.:—

TABLE X.

Compound.	Relative Rate of Hydrolysis.
α-Methyl glucoside . . .	100
β-Methyl glucoside . . .	179
α-Methyl galactoside . . .	542
β-Methyl galactoside . . .	884
Salicin	601
Maltose	740
Milk sugar	582

The rate of hydrolysis of the other disaccharides mentioned on p. 41 has not yet been determined, but it is known that trehalose is only hydrolysed with excessive difficulty and differs therefore markedly from cane sugar.

The relative strength of acids as measured by their inverting power is dependent on the nature of the sugar by means of which the comparison is made, and even with the same sugar the ratio is different at different temperatures. The following table compiled by Caldwell illustrates this point. It would, however, lead too far to discuss the significance of these observations here.

TABLE XI.

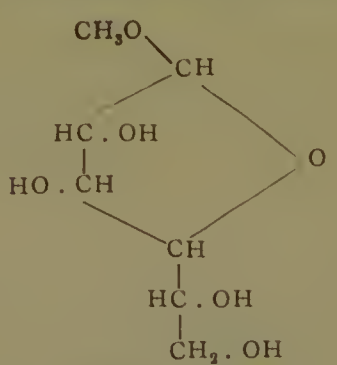
Sugar Hydrolysed.	Temp.	Relative Activities of the Acids.			
		HCl.	H_2SO_4.	$H_2C_2O_4$.	Camphor-Sulphonic (Reychler).
Sucrose	25°	100	53·7	18·2	89·8
Salicin	95°	100	49·9	23·3	—
Maltose	74°	100	40·5	14·1	—
Lactose	60°	100	47·7	—	68·6
(Conductivity) . . .	25°	100	61·9	19·7	—

The foregoing data (Table X.), though at present somewhat scanty, afford important material for the discussion of the nature of the hydrolytic process. Considering the hydrolysis of the glucosides two views are possible, either (1) that the compound behaves much as the simple ether $CH_3 . O . CH_3$ would, and that the hydrolyst becomes associated with the oxygen atom to which the CH_3 group is attached; or (2) that the attachment is to the oxygen atom in the ring. On the former view the two isomeric α- and β-glucosides should be hydrolysed with equal readiness as the methoxyl groups are equally weighted in the α- and β- positions.

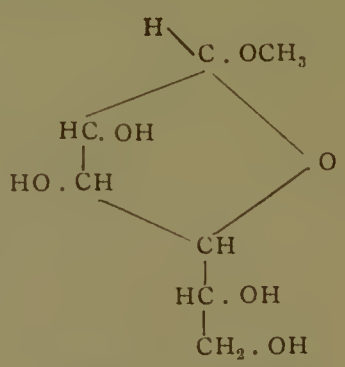
Actually in the case of both glucose and galactose the β-derivative is hydrolysed about 1·75 times as readily as the α-derivative, and, as there is every reason for thinking that the mechanism of change is the same in both cases, the difference in the rate of hydrolysis can only be due in main to the relative distances of the OCH_3 groups from the centre of change.

There is little doubt that the active system, within which change takes place, is formed by the association of acid-water molecules with the oxygen atom in the pentaphane ring. Oxonium compounds are formed of the type already discussed at length on pp. 18, 19. In other words, this oxygen is the centre from which attack proceeds.

Reference to a solid model will readily show that a distinct difference exists in the relative distances of the – OCH_3 group, when in the α- and β- positions, from the oxygen atom in the ring; this is but imperfectly rendered on a plane surface.



α-Methyl glucoside.



β-Methyl glucoside.

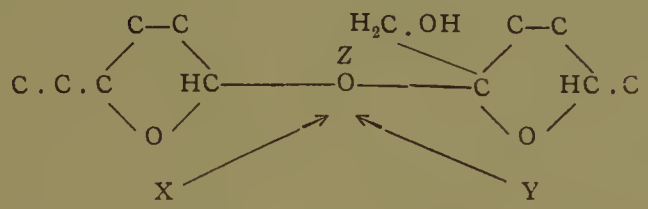
The α-methyl glucoside, since it is the most stable form, may be assumed to be that in which the methoxyl (OCH_3) group is furthest removed from the pentaphane oxygen as shown above: conversely, the β-glucoside will be that in which the methoxyl is nearest the oxygen centre.

It must be assumed in the case of the galactosides, which are more

readily hydrolysed than the glucosides, that the interchange in the position of the groups attached to the γ-carbon atom, which involves a shift in the position of the ring, brings the pentaphane oxygen nearer the methoxyl group (p. 13) and so facilitates action. It is impossible to represent such a change on a plane surface, but it will be readily understood on reference to the model.

The application of this line of argument to the disaccharides promises most interesting results.

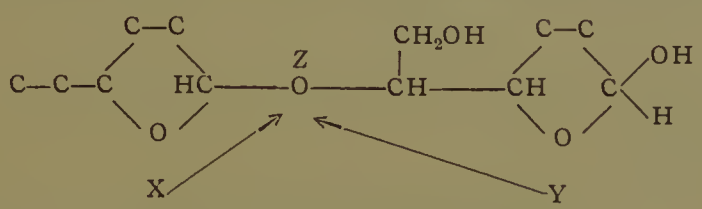
In cane sugar, for example, attack may be expected to proceed from *both* pentaphane oxygen centres, marked X and Y in the skeleton formula, towards the centre marked Z, at which scission of the molecule occurs :—



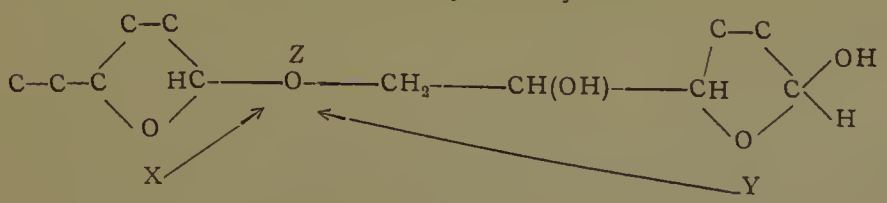
Glucose half of molecule. Fructose half of molecule.

In the cane sugar formula already assumed, these three centres are in the closest possible contiguity : everything is in favour of hydrolysis, which accordingly may be expected to take place with great rapidity.

As elsewhere pointed out (p. 42), two types of reducing disaccharides may be formulated according to whether the primary or secondary alcohol group of one sugar is joined to the glucoside half of the molecule. These types may be formulated in skeleton thus :—



Type A.—Secondary alcohol junction.



Type B.—Primary alcohol junction.

In disaccharides of type A, attack will proceed from centre X and to some extent from centre Y, though this is further removed from exercising influence than in the case of cane sugar.

In disaccharides of type B, centre Y is still further removed from

centre Z, and its influence may be supposed to be correspondingly weakened. Carbohydrates of this type will be least easily hydrolysed.

Differences introduced by the second hexose occupying the α- or β-positions will mainly effect the distance XZ in the formula, *e.g.*, in practice increase or decrease the magnitude of the attack from the centre X, but they will also have an effect on the nearness of the centres Y and Z. As before mentioned, these reasonings are best followed with the aid of a solid model.

It is possible on the basis of the foregoing argument to assign type formulæ to maltose and lactose, but it would be premature to do so until the rate of hydrolysis of their isomerides has been determined.

The laws of hydrolysis by enzymes have been dealt with by Bayliss (Monograph on Enzyme Action), and the details of their selective action towards the disaccharides will be found in Chapters IV. and V. of this monograph.

Enzymes are far more active as hydrolysing agents than acids, a very minute quantity at the ordinary temperature being far more powerful than very strong acid at a high temperature.

It is perhaps desirable here to lay emphasis on the difference noticeable in the behaviour of enzymes and acids respectively as hydrolytic agents. It is due mainly, if not wholly, (1) to the superior affinity of the enzymes for the carbohydrates; (2) to the very different behaviour of the two classes of hydrolysts towards water—which is a consequence of the colloid nature of the one and the crystalloid nature of the other.

The Synthesis of Monosaccharides by Chemical Means.—The synthetical preparation of natural dextroglucose from its elements may be justly claimed as one of the greatest achievements of the chemist, and it is enhanced in interest by the great biological importance of the carbohydrates.

In the following section a brief outline is given of the operations performed in preparing glucose and fructose from their elements. Dealing first with the earlier work, the first attempt which was in any way successful was that made by Butlerow, who showed that when trioxymethylene is condensed by means of lime water a syrupy substance is obtained which has the properties of a sugar. Subsequently Loew improved the technique of the method and named the product he obtained formose. Fischer and Tafel started with acrolein dibromide and effected condensation of this by means of baryta, the change being expressed by the equation :—

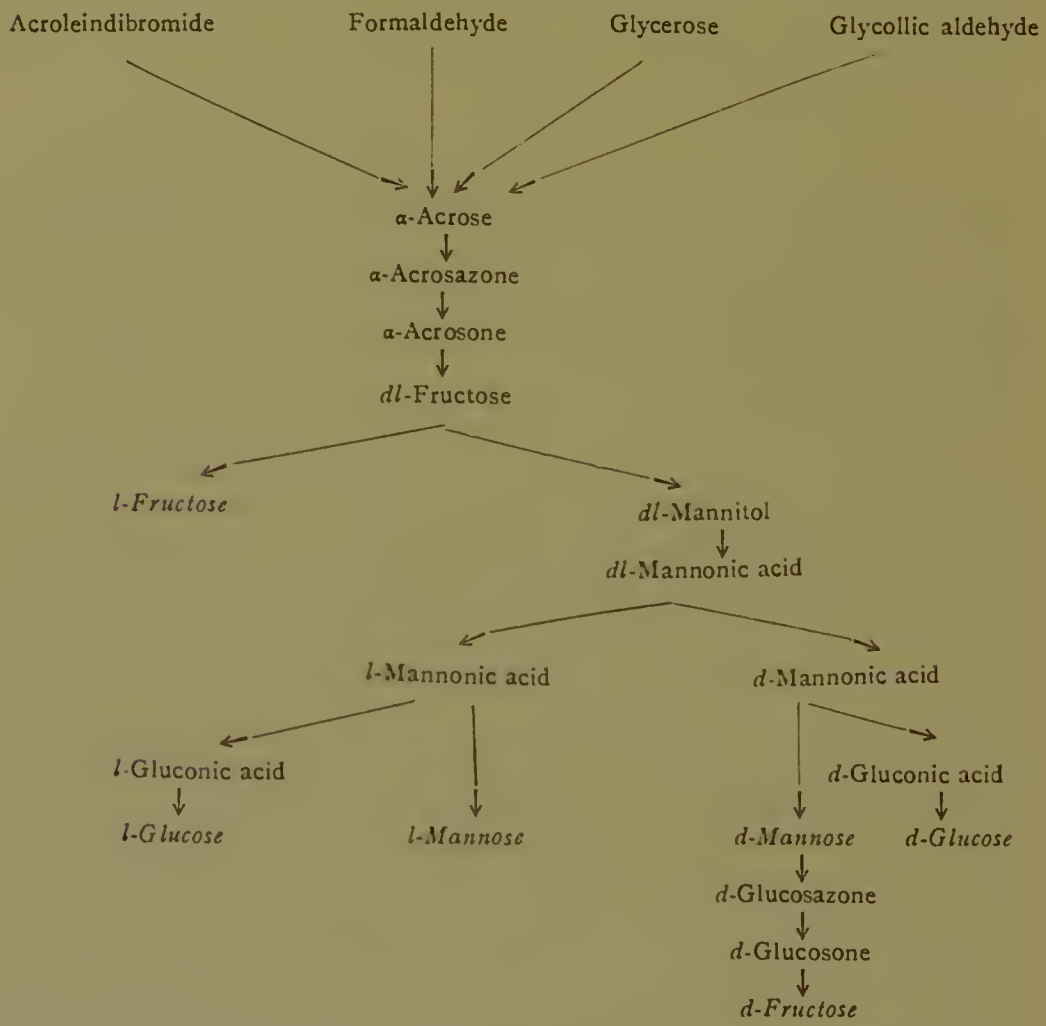
$$2C_3H_4OBr_2 + 2Ba(OH)_2 = C_6H_{12}O_6 + 2BaBr_2.$$

They showed that the syrupy product obtained contained two sugars distinguished as *a*- and *β*-acrose. Subsequently glycerose was made the starting-point for the synthesis; crude-glycerose is a mixture of glyceric aldehyde, $CH_2(OH).CH(OH).CHO$, and dihydroxyacetone, $CH_2(OH).CO.CH_2(OH)$, and these two compounds can be formulated as undergoing the "aldol" condensation forming a ketone, $CH_2(OH).(CH.OH)_3.CO.CH_2(OH)$, which has the same composition as fructose. *a*- and *β*-Acrose were obtained from this condensation and characterised by means of the osazones they formed with phenylhydrazine. *a*-Acrosazone was found to possess a remarkable resemblance to glucosazone differing only in being optically inactive. More recently Fenton has shown that glycollic aldehyde, $CH_2(OH).CHO$, may be used as the starting-point of the synthetical process; three molecules of it condense to *a*-acrose.

A product of synthesis by all these methods is *a*-acrose. Fischer converted this firstly into acrose phenyl osazone in order to isolate it from the mixture of substances and then into acrosone by treatment with hydrochloric acid as described in Chapter II. Acrosone, on reduction, yielded firstly a sweet syrup having all the properties of fructose, and secondly on further reduction an alcohol, *a*-acritol, very like mannitol but differing in being optically inactive. There was no doubt that *a*-acrose was inactive *dl*-fructose. The further problem was to obtain an optically active sugar from this. The product was partially fermented with yeast and a dextrorotatory sugar *l*-fructose was obtained, but this biological method did not lead to the isolation of the natural sugar. Indeed to obtain this a number of operations were necessary. *dl*-Fructose was reduced to *dl*-mannitol and the latter oxidised to the corresponding acid, *dl*-mannonic acid. (This acid forms a characteristic hydrazide from which it can be easily regenerated.) The racemic acid gave crystalline alkaloid salts and these were separated by fractional crystallisation; in this manner their resolution into the optically active forms was effected just as Pasteur did in the case of racemic tartaric acid. *d*- and *l*-Mannonic acids were thus obtained by the crystallisation of the strychnine or morphine salt of the synthetical racemic acid: by reduction of their lactones, they were converted into *d*- and *l*-mannose and the complete synthesis of these hexoses accomplished. To pass to *d*-fructose it only remained to reduce the mannosone (identical with glucosone) formed from *d*-mannose-phenyl osazone in the manner already described (compare Chap. II.).

The synthetical mannonic acids above mentioned are converted into the corresponding gluconic acids when heated with pyridine or

quinoline (see p. 25), and it was only necessary to reduce these acids to obtain the corresponding glucoses. The stages of these syntheses are summarised in the following chart:—



Proceeding in this way Fischer effected the synthesis of the six hexoses derived from mannitol, and extended the methods to the synthesis of a number of isomeric hexoses which do not occur naturally. To-day, out of the sixteen possible isomeric aldohexoses, according to the Le Bel-Van't Hoff theory, twelve have been prepared synthetically.

Theoretically a simpler method of passing from fructose (*a*-acrose) to glucose and mannose is afforded by warming with alkali, when the isomeric transformations observed by Lobry de Bruyn take place. These are of particular interest in the case of sorbose, which is converted into galactose and tagatose. Sorbose is derived from mannitol, galactose from dulcitol, so that this transformation connects the hexoses

derived from the two alcohols and indirectly effects the complete synthesis of all the sugars derived from dulcitol.

Before this transformation was discovered Fischer found it necessary to degrade gulonic acid to the pentose sugar xylose, transform this into the isomeric lyxose and combine lyxose with hydrogen cyanide to give galactonic acid. It was only in this somewhat roundabout fashion that the complete synthesis of galactose and other hexoses derived from dulcitol could be effected.

The other products of synthesis, β-acrose and formose, have not been further investigated. Fischer regarded both of them as containing a branched and not a straight chain of carbon atoms.

Both glycollic aldehyde and dioxyacetone are produced when formaldehyde is condensed by means of calcium carbonate.

The Synthesis of Monosaccharides in the Plant.—The synthesis of carbohydrates carried on by the green leaves of growing plants is the most fundamental of all biochemical syntheses, and its full explanation still baffles investigators.[1]

The first *visible* product of the process is starch (Sachs) and the first sugar as yet identified is cane sugar[2] (Brown and Morris). The hypothesis that formaldehyde, formed by the reduction of carbon dioxide, is the first product of assimilation, was advanced by Baeyer in 1870: the aldehyde is considered subsequently to undergo polymerisation to carbohydrate.

Although this hypothesis is generally accepted, the difficulty has always been experienced that all attempts to prove the presence of formaldehyde in the green parts of plants have led to inconclusive results and indeed indicated that it acts as a poison.

The supposed photosynthesis of formaldehyde outside the plant by Usher and Priestley has been severely criticised (Ewart, Mameli and Pollacci), but the most recent investigations support its correctness.

Glycollic and glyceric aldehydes and dihydroxyacetone are all intermediate stages in the laboratory synthesis of fructose from form-

[1] A full account of the historical side of the question has been given by Meldola in a presidential address to the Chemical Society in 1906.

[2] The conclusion of Brown and Morris that cane sugar is the first carbohydrate to arise in photosynthesis rather than glucose has been criticised adversely on the grounds that the sucrose may come from maltose formed from the leaf starch, and there is much in this objection. Cane sugar is formed when barley embryos are fed on maltose but not when they are fed on glucose, although in the latter case the plantlet is found to contain invert sugar (Brown and Morris). J. Parkin, working with the snowdrop, which does not produce starch in its mesophyll, interprets his results as showing that the formation of sucrose precedes that of the hexoses. He supports the view that the first sugar of photosynthesis arises in the chloroplast as a product split off from a complex protein aggregate.

aldehyde, but there is no evidence of these being found among normal plant products. They have so far only been encountered as down-grade products of the action of certain bacteria on mannitol or glucose. Attempts to imitate in the laboratory the formation of formaldehyde from carbon dioxide and water

$$H_2CO_3 + 2H_2O \rightarrow CH_2O + 2H_2O_2$$

have been numerous, but, if some controversial and very doubtful experiments be excepted, formic acid has been in all cases the sole product of the reduction. However, definite proof of the formation of formaldehyde has been recently given by Fenton (1907) who has shown that it is formed when carbon dioxide is reduced by means of metallic magnesium.

This observation of Fenton is deeply significant when considered in relation to Willstätter's recent discovery that chlorophyll contains magnesium as an integral part of the molecule. He regards the magnesium as playing just as important a rôle in the process of assimilation in plants as does the iron content of hæmoglobin in its function as oxygen carrier.

Assuming that Baeyer's hypothesis is correct and that formaldehyde is the first product of the synthesis, two questions await an answer. Firstly, how is the condensation of the aldehyde caused; secondly, through what immediate stages do the compounds pass?

The vital synthesis differs essentially from that carried out in the laboratory in affording optically active products. It might be supposed that the plant manufactures inactive racemic hexose and uses the laevo-isomerides for purposes which are still unknown. In spite of frequent search, however, it has never been possible to detect *l*-glucose or *l*-fructose in the leaves of plants, and the work of Brown and Morris leaves hardly any doubt that hexoses of the *d*-series and their polysaccharides are the only products of assimilation.

The living organism is not satisfied with merely elaborating a particular sugar, but shapes it in a definite manner to a definite space configuration.

Fischer has pictured the carbon dioxide or formaldehyde as entering into combination with the complicated optically active protoplasm of the chlorophyll granule, and being synthesised to optically active carbohydrates under the influence of the asymmetry of the protoplasm molecule.

The formaldehyde elements are received one after the other, and superposed according to a definite plan until six are united, when the

completed dextroglucose or fructose molecule is split off and the process begins anew, only optically active substances being formed. Synthesis by laboratory methods leads to optically inactive forms, though apparently chemical synthesis does not take place entirely symmetrically when several asymmetric carbon atoms are present. Fischer, for example, has failed to isolate any other racemic hexose than *a*-acrose (*β*-acrose and formose being considered to have branched chains) from the condensation of formaldehyde or glycerose, whereas had this synthesis been entirely symmetric, several isomerides should have been formed at the same time.

It is now generally agreed that the protoplasm of the chlorophyll granule contains enzyme elements, and that it is these which occasion synthesis. The protoplasmic complex may be regarded as built up of a series of associated templates (enzymes) which serve as patterns for the maintenance of vital processes and of growth. The assimilated carbon dioxide, either before or after condensation to formaldehyde, is brought into contact with these templates in the protoplasm, and contiguous molecules are united to form the complete sugar, shaped according to the structure of the template. The enzyme specific for each particular hexose when incorporated in the protoplasmic complex may well serve as the template for its manufacture. Maltase, for example, should occasion the formation of *a*-glucose, emulsin that of *β*-glucose, lactase that of galactose, and invertase, or some similar enzyme, that of fructose. The existence of contiguous maltase and invertase[1] branches in the protoplasmic complex might determine the formation of glucose and fructose in contiguity, and these might unite to cane sugar. Again two glucose molecules in contiguity might unite to maltose, or a series formed in contiguity might remain potentially active so that a number would unite and give rise to a starch molecule. *a*- and *β*-glucose would remain as such so long as they were incorporated with the protoplasm ; when split off into the cell fluid they would no doubt tend to pass over into the equilibrated mixture.

[1] Armstrong's recent researches suggest that invertase is compatible, at one and the same instant, with both glucose and fructose, so that its presence in the protoplasmic complex would, under suitable conditions, lead to the formation of cane sugar. As already noted (Chap. III.) Pottevin considers that fructose is compatible, not with invertase, but with a new enzyme.

The Synthesis of Disaccharides.—Although in the hands of Fischer the problem of the synthetical preparation of the natural simple carbohydrates—the monosaccharides—has been solved, the next step, the synthesis of the disaccharides, still awaits a satisfactory solution.

The oldest synthetical disaccharide was obtained by Fischer by the action of cold concentrated hydrochloric acid on glucose. The compound obtained was termed isomaltose on account of the resemblance to maltose, from which it differed in being nonfermentable. The process had the disadvantage that it could not be controlled so that only small quantities of disaccharide were formed together with considerable quantities of dextrin-like products. It was shown subsequently, as described later, that both maltose and isomaltose are formed by this process. A more hopeful method, based on Michael's glucoside synthesis, appeared to be the combination of acetochloro glucose with the sodium salt of a hexose. This method has been repeatedly used in attempting to synthesise cane sugar, and Marchlewski claimed to have been successful in artificially obtaining this sugar. Subsequent workers have found it impossible to confirm his results, and they are to be queried also for other reasons, chief of which is the observation of Fischer and Armstrong that α-compounds of glucose in presence of alkali undergo rearrangement to β-compounds. These observers failed to prepare α-phenyl glucoside from α-acetochloro glucose and sodium phenolate, obtaining instead the β-phenyl glucoside. Sucrose, a derivative of α-glucose, should not therefore be formed. The evidence brought forward by Marchlewski in proof of the formation of cane sugar was also very inadequate. There are thus no grounds for accepting this synthesis.

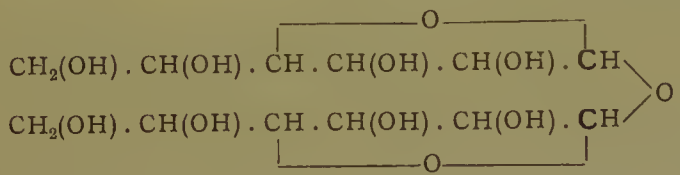
By the interaction of acetochloro galactose with sodium glucosate or of acetochloro glucose with sodium galactosate, Fischer and Armstrong obtained disaccharides of the type of maltose which they termed galactosido-glucose and glucosido-galactose. These sugars were sufficiently closely related to the natural products to be hydrolysed by enzymes. Top yeast was without action, bottom yeast was able to ferment both disaccharides. They were hydrolysed by emulsin but not affected by maltase or invertase. Both reduced Fehling's solution, formed phenyl osazones and osones, but could not be obtained in a crystalline state. The galactosido-glucose possessed very great similarity to the natural sugar melibiose both in structure, similarity of the phenyl and bromophenyl osazones and in physiological behaviour, and it is very probable both disaccharides are identical.

Quite recently Fischer and Delbrück have made use of β-aceto-

bromo glucose to effect the synthesis of disaccharides allied to trehalose. When acetobromo glucose is shaken in dry ethereal solution with silver carbonate and traces of water are added from time to time, bromine is eliminated and two molecules are joined through the intermediary of an oxygen atom to an octacetyl disaccharide $2C_{14}H_{19}O_9Br + H_2O = C_{28}H_{38}O_{19} + 2HBr$. This is obtained both crystalline and in an amorphous form, the latter being regarded as a mixture of isomerides.

The acetyl compounds when hydrolysed by cold barium hydroxide solution are converted into disaccharides. That from the crystalline acetate, termed isotrehalose, differs from trehalose in optical rotatory power $[\alpha]_D - 39{\cdot}4^\circ$, but resembles it closely in chemical properties. It is a colourless amorphous powder, which does not reduce Fehling's solution and is easily hydrolysed to glucose when boiled with dilute mineral acids. The disaccharide from the amorphous acetate is regarded as a mixture, it has $[\alpha]$ about $- 1{\cdot}3^\circ$. It is remarkable in being partially hydrolysed both by yeast extract and by emulsin.

Consideration of the constitutional formula of trehalose—



shows that three stereoisomerides are possible as the two carbons in clarendon type are asymmetric. Using the prefixes α and β in the same sense as in the acetobromo glucoses, these isomerides may be described as $\alpha\alpha$, $\beta\beta$ or $\alpha\beta$, according as the constituent glucoses are present in the α or β form. The behaviour of the new sugars towards enzymes may possibly be expected to give a clue to their structure.

Synthesis by Enzymes.—Far more interesting than the above method of synthesis is that effected by means of enzymes. There can be no doubt that, in the plant, enzymes function as synthetical agents.

The first to observe the synthetical or, as he termed it, reversible action of enzymes was Croft Hill. Hill proved that the hydrolysis of maltose by dried yeast extract in concentrated solutions was not complete, and that, starting from glucose alone in concentrated solution, a disaccharide was produced by the action of maltase. This sugar he at first considered to be maltose, a conclusion contraverted by Emmerling, who, repeating Croft Hill's experiments, considered the product to be *iso*maltose identical with that obtained by Fischer by the action of acid on glucose. Subsequently Croft Hill admitted the chief product to be an isomeride of glucose, but he regarded it as different from

isomaltose and termed it revertose. He still claimed that maltose is also formed in small quantity. E. F. Armstrong considers that the product of the synthetical action of maltase on glucose is *iso*maltose identical with that produced by the action of hydrochloric acid on glucose, and shows that the two products agree in being hydrolysed by emulsin though not by maltase. They are accordingly regarded as having the structure of glucose β-glucosides. Croft Hill showed that his synthetical product was almost completely hydrolysed on dilution, indicating that the process is reversible, or that at all events the same mixture of enzymes which effects synthesis is able to hydrolyse the synthetic product. An explanation of the removal of the isomaltose, a fact which it was at first somewhat difficult to bring into line, is perhaps afforded by the discovery of emulsin in yeast by Henry and Auld.

A disaccharide is also formed when a mixture of glucose and galactose in concentrated solution is left in contact with lactase. This is undoubtably isomeric with milk sugar but differs from it in being completely fermented by bottom yeast.

The process by which a monosaccharide is converted into a disaccharide in presence of a synthetical catalyst must be regarded as precisely similar to that by which α- and β-glucoses are converted into the two methyl-glucosides. Glucose on condensation should give rise to both maltose and isomaltose synthesised from α- and β-glucose respectively. The proportion of each ultimately present in the equilibrium will depend to some extent on the proportions of the two glucoses in their equilibrated mixture and on their (possibly unequal) rates of condensation. This reasoning should apply so long as the condensation is uncontrolled. Inasmuch as hydrolysis under the influence of enzymes is an absolutely selective process, as opposed to hydrolysis by acids which is general in character, it is to be supposed that synthesis under the influence of enzymes is likewise a controlled operation.

The proof that hydrochloric acid forms both *iso*maltose and maltose from glucose was first given by E. F. Armstrong. The method of purification of the synthetical isomaltose mixture adopted by Fischer, *viz.*, fermentation of the neutralised product with brewers' yeast, would have destroyed any maltose which had been formed. Armstrong fermented a portion of the product with *S. Marxianus*, a yeast which does not contain maltase and therefore is without action on maltose, in order to destroy the unchanged glucose. The resulting solution contained both maltose and *iso*maltose, and was partially

hydrolysed by both maltase and emulsin. To remove the *iso*maltose it was submitted to the joint action of emulsin and *S. Marxianus*. It was not found possible to obtain the maltose in a crystalline condition from this solution, but the character of the osazone formed and the biological behaviour of the sugar leave little doubt of the presence of this sugar. Another portion of the original synthetical sugar was fermented with *S. intermedians*, and so freed from glucose and maltose. The resulting *iso*maltose solution behaved in all respects as described by Fischer.

The manner of the synthesis by enzymes is still a matter of dispute. It is urged on the one hand that enzymes produce by synthesis the same bodies which they hydrolyse; on the other hand, it is suggested that the action of the enzyme is restricted to the formation of a compound isomeric with that normally hydrolysed by the enzyme. A third view is that altogether distinct enzymes effect synthesis.

The arguments in favour of accepting the first view have been clearly put by Bayliss (see the Monograph on Enzyme Action in this series), and need not be repeated here.

The question is complicated by the fact that the catalysts used are all mixtures of several enzymes. Yeast extract (maltase) contains at least five sucroclasts; emulsin, according to a recent work, at least three.

Armstrong has shown that the main product in the case of the action of yeast extract on glucose is isomaltose; in the case of emulsin the main product is maltose. Whilst it could not be definitely asserted that the isomerides were not also formed, their amount in any case must have been small.

Bayliss' contention that, if the synthetic body is incapable of being hydrolysed by the enzymes present, action should continue until all the glucose is converted into disaccharide, does not sufficiently take into account the equilibrium resulting from the combination of enzyme and sugar and the great retarding influence of glucose—both thoroughly established facts. Further, each molecule of disaccharide formed liberates a molecule of water, thereby diluting the solution and lessening the opportunity for synthetic action. Lastly, enzyme extracts, unlike inorganic catalysts, do not remain of constant strength.

It is difficult to attribute the formation of isomaltose in Croft Hill's experiments entirely to emulsin. The amount in brewers' yeast is but small; Henry and Auld demonstrated its presence under very special conditions. A cold-water extract of the dried yeast used by

the writer has never been found to have any hydrolytic action on β-methyl glucoside.

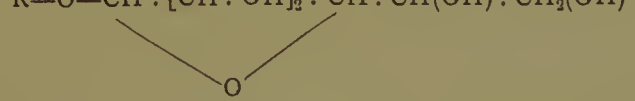
The question cannot be discussed here at greater length, but obviously much experimental work remains to be done before it can be settled. The fact that enzymes bring about a controlled synthesis of disaccharides is, however, clearly established. Under natural conditions apparently only one product is formed, as there is no evidence that isomaltose, for example, is ever present in the plant. On the other hand, emulsin is widely distributed, maltase of very rare occurrence.

The third view that synthesis and hydrolysis are effected by different enzymes, though not overlooked by earlier workers, has been brought into prominence by the recent experimental work of Rosenthaler. Emulsin in presence of hydrogen cyanide and benzaldehyde brings about the formation of optically active benzaldehyde cyanohydrin, a substance which it also hydrolyses. Saturation of the enzyme solution with magnesium sulphate or half-saturation with ammonium sulphate produces a precipitate which is soluble in water. The filtrate has no synthetic activity, but is able to effect hydrolysis as before; the precipitate possesses synthetic activity and some hydrolytic activity. It is considered by Rosenthaler that emulsin consists of two distinct enzymes, one promoting synthesis, the other causing hydrolysis of benzaldehyde cyanohydrin.

CHAPTER VII.

THE NATURAL AND SYNTHETIC GLUCOSIDES.

THE term glucoside is applied to a large number of bodies having the property in common of furnishing a *glucose* and one or more other products when hydrolysed by acids. They are resolved with the addition of the elements of water into simpler compounds. Representatives of nearly every class of organic compound occur in plants, chiefly in the fruit, bark and roots, in combination with a sugar which is in most cases dextroglucose. These compounds are glucose ethers of alcohols, acids, phenols, etc.; they correspond in structure to the simple methyl glucosides, and the general formula of a glucoside is accordingly written—



where R represents the organic radicle. It is noteworthy that the vegetable bases are only seldom found in the form of glucosides.

The glucosides correspond to a certain extent to the paired glucuronic acid derivatives previously mentioned. In both instances more or less reactive specific substances are combined with the sugar residue to form indifferent and frequently more soluble substances.

Glucosides are obtained by extraction of the plant substance with water or alcohol, an operation often conveniently performed in a Soxhlet apparatus. It is necessary in the majority of cases first to destroy the accompanying enzyme when water is used as solvent. If this operation be omitted the glucoside is destroyed in the process of extraction. The purification of the extract is often a matter of difficulty owing to the scanty proportion of glucoside present.

The glucosides as a class are generally colourless crystalline solids, having a bitter taste and laevorotatory optical power. Some of the best-known glucosides are the amygdalin of the almond and other rosaceous plants, the salicin of the willow and the sinigrin of the cruciferæ.

The glucosides are all hydrolysed by heating with mineral acids to sugar and an organic residue. They are decomposed at very different rates, some glucosides (*e.g.*, gynocardin) being extremely resistant to acid hydrolysis.

In the majority of cases the glucosides are hydrolysed by enzymes. The appropriate enzyme is contained in the same plant tissue, but in different cells, gaining access to the glucoside only when the tissue is destroyed. A great number of such enzymes exist, but it is too much to say that each glucoside has a special enzyme for its decomposition. The best-known glucoside splitting enzymes are the emulsin of almonds and the myrosin of black mustard seeds. Both these enzymes can effect hydrolysis of a number of glucosides.

Emulsin is especially wide in its action. Since it is the specific enzyme for β-alkyl glucosides, all glucosides hydrolysed by it are regarded as derivatives of β-glucose, though the possibility that emulsin is a mixture of enzymes must not be lost sight of.

The hydrolysis of glucosides by myrosin is undoubtedly connected with their sulphur content.

Most of the known glucosides are derived from dextroglucose. Others are derivatives of rhamnose or of galactose. In many cases the exact nature of the sugar has yet to be determined. Glucosides containing rhamnose likewise require a special enzyme to effect their hydrolysis.

Some glucosides yield two or more sugar molecules on hydrolysis. In such cases the sugar molecules are united as di- or trisaccharides. Using appropriate enzymes, the sugar groups may be removed one at a time, and new glucosides are formed. Thus amygdalin contains two glucose residues, one of which is removed by an enzyme present in yeast and termed amygdalase. The new glucoside so formed is named mandelonitrile glucoside.

Both on account of the very small quantity of a glucoside usually present in a plant, and the fact that glucosides do not as a rule form insoluble characteristic derivatives which allow of their isolation, it is difficult to discover new glucosides and still more so to determine their nature. The introduction of biochemical methods has much facilitated work of this kind. Bourquelot's biological method has led to the discovery of several new glucosides, and ter Meulen has established the nature of the sugar component in several instances. Ter Meulen makes use of the fact (p. 57) that an enzyme is only compatible with and therefore only enters into combination with that sugar, the simple glucosidic compounds of which it is able to hydrolyse.

He has investigated the rate of hydrolysis of a glucoside by the appropriate enzyme in presence of a number of the simple sugars. Only one of these sugars retards the change; the others are almost without influence. The glucoside in question is considered to be a derivative of that sugar which retarded the hydrolysis.

For instance, rhamninose alone retards the hydrolysis of xanthorhamnin; glucose alone retards the decomposition of salicin or of amygdalin. In the case of glucosides of which the nature of the sugar component was not absolutely established, it was shown that aesculin, arbutin, coniferin, indican, sinigrin and several other glucosides containing mustard oils are derivatives of *d*-glucose.

Bourquelot's biological method of examining plants for glucosides consists in the addition of emulsin to an extract of the plant and the determination of the changes in optical rotation and cupric reducing power after a period of incubation. A change indicates the presence of β-glucosides and its magnitude gives a rough indication of their quantity.

In this manner taxicatin, $C_{13}H_{22}O_7$, has been discovered in *Taxus baccata* (Lefebvre) and the presence of aucubin demonstrated in a number of species of plantago (Bourdier).

The use of invertase in the same manner affords a test for the presence of sucrose or raffinose.

A number of the better-known glucosides are given in the following table which also shows the products of hydrolysis. They are classified under alcohols, phenols, aldehydes, etc., according to the nature of the non-sugar part of the molecule (see Table XII., p. 80).

The better-known glucosidoclastic enzymes are grouped in Table XIII. together with the glucosides they decompose. Emulsin hydrolyses arbutin, salicin, coniferin, syringin, helicin, amygdalin, mandelonitrile glucoside, sambunigrin, prulaurasin, dhurrin, aesculin, daphnin and calmatambin. It is without action on phloridzin, populin, gaultherin, quercitrin and sinigrin.

TABLE XIII.

GLUCOSIDOCLASTIC ENZYMES.

Enzyme.	Hydrolyses.
Emulsin	Many natural glucosides Synthetical β-glucosides
Myrosin	Sinigrin and sulphur glucosides
Rhamnase	Xanthorhamnin
Gaultherase	Gaultherin
Tannase	Tannins
Lotase	Lotusin

TABLE XII.

Natural Glucosides.

Glucoside.		Products of Hydrolysis.
		Phenols.
Arbutin	$C_{12}H_{16}O_7$	Glucose + hydroquinone
Methyl arbutin	$C_{13}H_{18}O_7$	Glucose + hydroquinone methyl ether
Phloridzin	$C_{21}H_{24}O_{10}$	Glucose + phloretin
Glycyphyllin	$C_{21}H_{24}O_9$	Rhamnose + phloretin
Hesperidin	$C_{50}H_{60}O_{27}$	Rhamnose + 2 glucose + hesperetin
Naringin		Rhamnose + glucose + narigenin
Iridin	$C_{24}H_{26}O_{13}$	Glucose + irigenin
Baptisin	$C_{26}H_{32}O_{14}$	Rhamnose + baptigenin
		Alcohols.
Salicin	$C_{13}H_{18}O_7$	Glucose + saligenin
Populin	$C_{20}H_{22}O_8$	Glucose + benzoylsaligenin
Coniferin	$C_{16}H_{22}O_8$	Glucose + coniferylalcohol
Syringin	$C_{17}H_{24}O_9$	Glucose + syringenin
		Aldehydes.
Helicin	$C_{13}H_{16}O_7$	Glucose + salicylaldehyde
Salinigrin	$C_{13}H_{16}O_7$	Glucose + *m*-oxybenzaldehyde
Amygdalin	$C_{20}H_{27}O_{11}N$	2 Glucose + *l*-mandelonitrile
Mandelonitrile glucoside	$C_{14}H_{17}O_6N$	Glucose + *l*-mandelonitrile
Sambunigrin	$C_{14}H_{17}O_6N$	Glucose + *d*-mandelonitrile
Prulaurasin	$C_{14}H_{17}O_6N$	Glucose + racemic mandelonitrile.
Dhurrin	$C_{14}H_{17}O_7N$	Glucose + *p*-oxymandelonitrile
		Acids.
Convolvulin	$C_{54}H_{86}O_{27}$	Glucose + rhodeose + convolvulinolic acid
Jalapin	$C_{34}H_{56}O_{16}$	Glucose + jalapinolic acid
Strophantin	$C_{49}H_{76}O_{19}$	Rhamnose + mannose + strophantidin
Gaultherin	$C_{14}H_{18}O_8$	Glucose + methylsalicylate
		Oxycumarin Derivatives.
Aesculin	$C_{15}H_{16}O_9$	Glucose + aesculetin
Daphnin	$C_{15}H_{16}O_9$	Glucose + daphnetin
Fraxin	$C_{16}H_{18}O_{10}$	Glucose + fraxetin
		Oxyanthraquinone Derivatives.
Ruberythrinic acid	$C_{26}H_{28}O_{14}$	Glucose + alizarin
Rubiadin glucoside	$C_{21}H_{20}O_9$	Glucose + rubiadin
Frangulin	$C_{21}H_{20}O_9$	Rhamnose + emodin.
		Oxyflavone Derivatives.
Apiin	$C_{27}H_{30}O_{15}$	Apiose + apigenin
Fustin	$C_{36}H_{26}O_{14}$	Rhamnose + fisetin
Quercitrin	$C_{21}H_{22}O_{12}$	Rhamnose + quercetin
Sophorin	$C_{27}H_{30}O_{16}$	Rhamnose + glucose + sophoretin
Xanthorhamnin	$C_{34}H_{42}O_{20}$	2 Rhamnose + galactose + rhamnetin
		Indoxyl Derivatives.
Indican	$C_{14}H_{17}O_6N$	Glucose + indoxyl
		Mustard Oils.
Sinigrin	$C_{10}H_{16}O_9NS_2K$	Glucose + allyl mustard oil + $KHSO_4$
Sinalbin	$C_{30}H_{42}O_{15}N_2S$	Glucose + sinapine acid sulphate + acrinyl-isothiocyanate
Glucotropaolin	$C_{14}H_{13}O_9NS_2K$	Glucose + benzyl mustard oil + $KHSO_4$
		Various.
Saponins		Glucose + galactose + sapogenins
Digitonin	$C_{54}H_{92}O_{28}$	Glucose + galactose + digitogenin
Digitalin	$C_{35}H_{56}O_{14}$	Glucose + digitalose + digitaligenin
Saponarin	$C_{21}H_{24}O_{12}$	Glucose + vixetin
Calmatambin	$C_{19}H_{28}O_3$	Glucose + calmatambetin

The Principal Glucosides.

Arbutin, a colourless, bitter, crystalline substance, is obtained, together with methyl arbutin, from the leaves of the bear berry, a small evergreen shrub (*Arbutus uva ursi*), and yields hydroquinone and glucose when hydrolysed by means of emulsin or mineral acids :—

$$C_{12}H_{16}O_7 + H_2O = C_6H_{12}O_6 + C_6H_6O_2.$$

Hydroquinone is a powerful antiseptic : hence the pharmacological value of arbutin, which has also a diuretic action. Methyl arbutin is one of the few glucosides which have been artificially synthesised. Michael prepared it by the interaction of hydroquinone methyl ether and acetochloro glucose.

Phloridzin, which is found in the bark of apple, pear, cherry, plum and other rosaceous trees, is remarkable for the property it possesses of causing glucosuria when taken internally. Emulsin is without action on it : mineral acids form glucose and phloretin, $C_{15}H_{14}O_5$, which is a condensation product of *p*-oxyhydratropic acid and phloroglucinol.

Phloridzin has the formula—

$$(C_6H_{11}O_5 . O)\,(OH)_2C_6H_2 . CO . CHMe . C_6H_4(OH).$$

Salicin, a colourless, crystalline, bitter substance, is the active constituent of willow bark ; it has long been used as a remedy against fever and in cases of acute rheumatism. It is hydrolysed by emulsin to glucose and saligenin (*o*-oxybenzyl alcohol), and has the formula $(C_6H_{11}O_5 . O) . C_6H_4 . CH_2OH$. Saligenin yields salicylic acid on oxidation, but has the advantage of being less irritant than this acid or its salts, and therefore does not produce digestive disturbances when administered medicinally.

Salicin forms another glucoside *helicin* (glucosalicylic aldehyde) on oxidation with dilute nitric acid. Helicin was obtained synthetically (from salicylic aldehyde) by Michael. It is hydrolysed by emulsin.

Populin, the benzoyl ether of salicin, is found in the bark of a number of species of poplar (*Populus*). It may be obtained from or converted into salicin. It is not hydrolysed by emulsin.

Salinigrin is closely related to salicin, yielding glucose and *m*-hydroxybenzaldehyde on acid hydrolysis. It was only found in one species (*Salix discolor*) out of thirty-three samples of willow and poplar examined by Jowett and Potter, all of which contained salicin.

Coniferin, the glucoside of the fir-tree, is of importance as the starting-point for the synthesis of vanillin which is formed from it by oxidation with chromic acid.

It yields glucose and coniferyl alcohol when hydrolysed by emulsin, and has the formula :—

$$(C_6H_{11}O_5 . O) . C_6H_3(OMe) . CH : CH . CH_2OH.$$

By careful oxidation glucovanillin is formed, and this may be oxidised to glucovanillic acid or reduced to glucovanillyl alcohol. All three glucosides are hydrolysed by emulsin.

A methoxy coniferin is *syringin*, the glucoside of the Syringa, which is likewise hydrolysed by emulsin to syringenin (methoxy coniferyl alcohol).

Amygdalin is perhaps the best known and at the same time the most interesting of the glucosides; it has formed the subject of repeated and fruitful investigation ever since the discovery seventy-nine years ago, and even to-day the exact structure is not satisfactorily established. It is an example of a glucoside which contains nitrogen; on hydrolysis it yields benzaldehyde, hydrogen cyanide and two molecules of glucose. It is found in large quantities in bitter almonds and in the kernels of apricots, peaches, plums and most fruits belonging to the Rosaceæ. It is the antecedent of the so-called essence of bitter almonds, and is widely used as a flavouring material. Like most glucosides it is a colourless, crystalline, bitter substance soluble in water.

The presence of hydrogen cyanide in the aqueous distillate of bitter almonds was observed at the very beginning of the nineteenth century by Bohm; the crystalline glucoside was first obtained by Robiquet and Boutron Charlard in 1830, who showed its connection with the essence of bitter almonds.

In 1837 Liebig and Wöhler found that amygdalin was hydrolysed by a certain nitrogenous substance, also existing in the almond, to which they gave the name emulsin, in accordance with the equation—

$$\underset{\text{Amygdalin.}}{C_{20}H_{27}O_{11}N} + 2H_2O = \underset{\text{Benzaldehyde.}}{C_7H_6O} + \underset{\text{Hydrogen cyanide.}}{HCN} + \underset{\text{Glucose.}}{2C_6H_{12}O_6}$$

They proved it to be a glucoside of benzaldehyde cyanhydrin.

Ludwig in 1856 pointed out that hot mineral acids hydrolyse amygdalin, giving rise to the same products as emulsin does. Schiff was the first to suggest that the two glucose molecules were united as a biose—

$$C_6H_5 . CH(CN) . O - C_6H_{10}O_4 . O . C_6H_{11}O_5,$$

and this view became generally accepted when it was shown by Fischer that amygdalin may be resolved by an enzyme, contained in

yeast extract, into a molecule of glucose and one of a new glucoside which he termed mandelonitrile glucoside—

$$C_6H_5 . CH(CN) . O—C_6H_{11}O_5.$$

Fischer came to the guarded conclusion that amygdalin was a derivative either of maltose or of a closely related diglucose. The view that amygdalin is a maltoside has passed into the literature (*cf.* Dunstan and Henry, British Association Report, York, 1906).

Recent work, however, does not support this supposition. Neither in its behaviour towards enzymes nor in its chemical properties does amygdalin behave as a maltoside.

When hydrolysed by means of strong hydrochloric acid, amygdalin gives *l*-mandelic acid, and Fischer's amygdonitrile glucoside is correspondingly *l*-mandelonitrile glucoside.

Amygdalin at first sight seems to present an exception to the rule that enzymes which attack β-glucosides are strictly without action on α-glucosides, and *vice versâ*. Emulsin hydrolyses amygdalin at both glucose junctions; an enzyme in yeast extract (maltase?) also attacks one of these. This junction must either be attackable by two distinct enzymes, or the enzymes in question must be mixtures and contain a common constituent. The latter hypothesis has proved to be correct.

Caldwell and Courtauld, in the course of a quantitative study of the hydrolysis of amygdalin by acids, showed that change takes place more readily at position Y in the molecule than at position X, as indicated in the formula, $C_6H_5 . \underset{X}{CH(CN)O} . C_6H_{10}O_4 . \underset{Y}{O} . C_6H_{11}O_5$. The first product of acid hydrolysis is therefore the mandelonitrile glucoside obtained by Fischer; and this can be prepared in such manner. It was further shown that the action of yeast extract on amygdalin was due not to maltase but to the presence of a hitherto unknown enzyme appropriately termed amygdalase. This is more stable towards heat than maltase, and can be obtained almost free from maltase by preparing the extract at an elevated temperature.

The fact that an enzyme distinct from maltase effects the hydrolysis of amygdalin is clear proof that the glucoside does not contain maltose. Additional confirmation of this is afforded by the fact that the rate of hydrolysis of amygdalin either by amygdalase or by emulsin (ter Meulen) is not affected by the presence of maltose. This last sugar should have slowed the reaction had it been a constituent of the glucoside.

When amygdalin is hydrolysed by emulsin it is not possible at any stage of the reaction to detect the presence of a diglucose. In

reality, under the influence of emulsin prepared from an aqueous extract of almonds, two actions are going on at the same time, *viz.*, hydrolysis at the centre Y, forming mandelonitrile glucoside and glucose, and, more slowly, hydrolysis of the mandelonitrile glucoside at X, forming benzaldehydecyanhydrin and glucose. By interrupting the hydrolysis at the proper point it is possible to isolate the mandelonitrile glucoside. Such experiments prove that almond extract contains amygdalase in addition to the emulsin proper, which hydrolyses β-glucosides. Amygdalase is entirely without action on β-glucosides.

The amygdalin molecule is exceptional in containing several centres, marked X, Y, Z in the formula, $\underset{Z}{NC}.CHPh.\underset{X}{O}.C_6H_{10}O_4.\underset{Y}{O}.C_6H_{11}O_5$, totally different in their chemical nature, which are attackable by hydrolytic agents; its behaviour is, therefore, of the very greatest interest.

Amygdalin yields the same products (glucose, benzaldehyde and hydrocyanic acid) when treated with emulsin as when heated with dilute hydrochloric acid. In each instance the primary formation of *l*-mandelonitrile glucoside indicates that the biose junction Y is the first point to be attacked. The course of hydrolysis by concentrated acids is altogether different (Walker and Krieble). Concentrated hydrochloric acid hydrolyses it to amygdalinic acid and ammonia in the first place at centre Z; subsequently, the amygdalinic acid breaks down at junction Y to *l*-mandelic acid glucoside and glucose so that junction X is the last point to be attacked. Concentrated sulphuric acid has very little tendency to attack the nitrile group at Z, the primary action being to eliminate *l*-mandelonitrile. The biose junction Y is the point most susceptible of attack by sulphuric acid at all concentrations. Sulphuric acid decomposes benzaldehyde cyanohydrin (junction Z) only with extreme difficulty.

In addition to *l*-mandelonitrile glucoside two other glucosides having the same composition are known. These are: prulaurasin, first described in the amorphous state under the name laurocerasin, and since obtained crystalline from the cherry laurel by Hérissey; and sambunigrin, separated by Bourquelot and Hérissey from the leaves of the common elder (*Sambucus niger*). These substances are both mandelonitrile glucosides; their properties are set out in the following table:—

TABLE XIV.

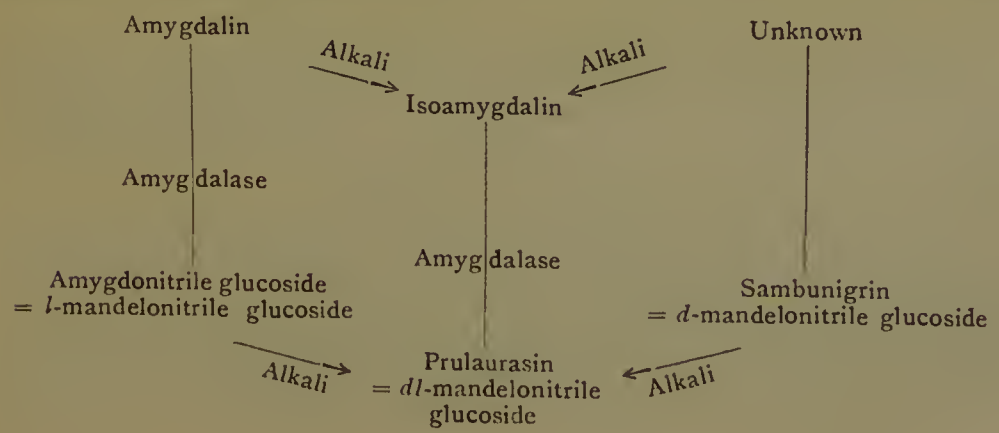
	M.-p.	$[\alpha]_D$.
Amygdonitrile glucoside = laevo mandelonitrile glucoside .	147-150°	−26·9°
Prulaurasin = racemic mandelonitrile glucoside . . .	120-122°	−52·7°
Sambunigrin = dextro mandelonitrile glucoside . . .	151-152°	−76·3°

Dunstan and Henry suggested that the differences between these lay in the nature of the sugar residue. This can hardly be the case, as they are all three attacked by emulsin, and therefore derivatives of β-glucose.

Prulaurasin is, in fact, a racemic mixture of the two stereoisomeric *d*- and *l*-mandelonitrile β-glucosides, and analogous to isoamygdalin, the racemic form of amygdalin, first prepared by the action of alkali on amygdalin by Walker and subsequently studied by Dakin, which yields inactive mandelic acid when hydrolysed by acids; indeed, prulaurasin is obtained by acting on isoamygdalin with yeast extract—amygdalase (Hérissey). *Sambunigrin* is the β-glucoside of *d*-mandelonitrile glucoside, and derived from a still unknown isomeride of amygdalin. Prulaurasin is obtained from either of the other two isomerides, when their aqueous solutions are rendered slightly alkaline.

The true relationship of these glucosides was first established by Caldwell and Courtauld, and their conclusions have been entirely confirmed by Bourquelot and Hérissey. More recently amygdonitrile glucoside has been discovered as a natural product, so that all three isomerides must play some part in plant economy. Hérissey found it in the young branches of *Cerasus Padus*; Power and Moore have obtained it from wild cherry bark (*Prunus serotina*).

The inter-relationship of these compounds is indicated in the accompanying scheme. Possibly the unknown isomeride of amygdalin will also be found in the plant:—



As mentioned above ordinary amygdalin, or as Walker terms it *l*-amygdalin, is converted rapidly at the ordinary temperature by alkali into a much more soluble substance which yields racemic mandelic acid when hydrolysed, together with a slight excess of dextro-mandelic acid. The simplest assumption that can be made regarding this change is that it consists only in the racemisation of the mandelic asymmetric carbon atom. Recent experiments of Walker and Krieble suggest, however, that other changes take place during racemisation, particularly when the solution is evaporated to dryness and so subjected to protracted heating. Apparently the new product formed is stable towards emulsin, and it is suggested that an intramolecular change from a β- into an α-glucoside has taken place creating a new isomeride of amygdalin. Amygdalin does not part with a glucose radicle when racemised and heated, nor is it hydrolysed to the ammonium salt of amygdalinic acid to any great extent. If confirmed, this transformation of β- into α-glucoside is of a very remarkable character.

Cyanogenetic Glucosides.—Hydrocyanic acid has frequently been isolated from plants, but it is only quite recently that its formation has been ascribed invariably to the decomposition of a glucoside. Besides amygdalin and the isomeric mandelonitrile glucosides a number of other glucosides have been isolated, yielding hydrogen cyanide when hydrolysed; they are conveniently grouped together under the term cyanogenetic glucosides. Although rare compared with the occurrence of saponin in plants the distribution of hydrogen cyanide is proving much wider than was at one time imagined; its production has been observed in many plants of economic importance. A useful list of plants which yield prussic acid has been compiled by Greshoff. Some of the cyanogenetic glucosides may be briefly mentioned.

Dhurrin, first isolated by Dunstan and Henry from the leaves and stems of the great millet, is a *para*-hydroxymandelonitrile glucoside, and therefore closely related to the three mandelonitrile glucosides just described. Like them it is hydrolysed by emulsin.

Gynocardin, isolated by Power from the oleaginous seeds of *Gynocardia odorata*, yields prussic acid, glucose and an unknown substance, $C_6H_8O_4$, on hydrolysis. It is accompanied in the seeds by an enzyme, gynocardase, which also decomposes amygdalin.

Linamarin or Phaseolunatin was first isolated by Jorissen and Hairs from young flax plants and subsequently by Dunstan and Henry from *Phaseolus lunatus*. The latter authors consider it to be acetonecyanohydrine-α-glucoside, and it is interesting as the first-known derivative of α-glucose, apart from maltose, occurring naturally. The beans of

Phaseolus lunatus contain two enzymes—an emulsin which, however, according to Dunstan, is without action on phaseolunatin and an enzyme of the maltase type which hydrolyses both phaseolunatin and amygdalin, forming mandelo-nitrile glucoside in the latter case. It is perhaps identical with the amygdalase described by Caldwell and Courtauld.

Lotusin discovered by Dunstan and Henry in *Lotus arabicus* is of interest for two reasons. Like amygdalin it gives rise to two molecules of dextrose on hydrolysis and therefore probably contains a disaccharide. The other products of hydrolysis are prussic acid and lotoflavin—an isomeride of fisetin. In the alkaline hydrolysis one of the dextrose residues is obtained as heptagluconic acid, indicating that the cyanogen radicle is associated with the sugar residue. Lotusin is not hydrolysed by almond emulsin but it is resolved by an enzyme (lotase) which accompanies it, but as this also decomposes amygdalin and salicin it probably contains emulsin.

Mustard Oil Glucosides.—A number of plants belonging to the cruciferæ yield glucosides containing sulphur. These give rise to mustard oils when hydrolysed by the enzyme myrosin which accompanies them in the plant. The best-known representatives of this class are sinigrin and sinalbin, found in the seeds of the black and white mustard. When the seed of black mustard is bruised and moistened, the odour of allylsulphocyanate is easily recognised. The myrosin and the glucoside are contained in separate cells in the seed, and do not interact until brought together by the solvent.

The recognition of an ethereal oil as the active principle of black mustard dates from 1730 (Boerhave). Bussy was the first to isolate the glucoside, which he termed potassium myronate, and the accompanying enzyme myrosin. Will and Körner gave the name sinigrin to the glucoside, and showed that it is hydrolysed to allylsulphocyanide, glucose and potassium hydrogen sulphate.

$$C_{10}H_{16}O_9NS_2K + H_2O = C_3H_5 . CNS + C_6H_{12}O_6 + KHSO_4$$

Sinigrin was subsequently investigated in detail by Gadamer, who proposed the formula —

$$C_3H_5 . N : C(S . C_6H_{11}O_5) . O(SO_2K).$$

It is not hydrolysed by emulsin or by yeast extract or any known enzyme other than myrosin. As hydrolysis proceeds, the increasing quantity of acid potassium sulphate formed renders the ferment less active and ultimately stops its action.

Guignard has very carefully investigated the localisation of myrosin in the plant. It occurs in special cells with finely granular contents which are free from starch, chlorophyll, fatty matter and aleurone grains.

Sinalbin is likewise hydrolysed by myrosin, which accompanies it in the seeds, to glucose, sinalbin mustard oil and acid sinapin sulphate—

$$C_{30}H_{42}O_{15}N_2S_2 + H_2O = C_6H_{12}O_6 + C_7H_7O \cdot NCS + C_{16}H_{24}O_5N \cdot HSO_4.$$

Indican.—Plants which yield indigo do not contain the colouring matter present as such but in the form of a glucoside indican, which is readily extracted from the leaf by means of acetone. Indican yields glucose and indoxyl on hydrolysis ; the indoxyl (colourless) undergoes further oxidation to indigotin (the blue colouring matter)—

$$\underset{\text{Indican.}}{C_{14}H_{17}O_6N} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Indoxyl.}}{C_8H_7ON} \qquad \underset{\text{Indoxyl.}}{2C_8H_7ON} + O_2 = 2H_2O + \underset{\text{Indigotin.}}{C_{16}H_{10}O_2N_2}$$

Indigotin is readily obtained on hydrolysing indican with dilute acids containing a little ferric chloride as an oxygen carrier, but the yield under these conditions is not quantitative.

Indican is also hydrolysed by a specific enzyme indimulsin, which is present in the leaves of the indigo plant. Emulsin also slowly hydrolyses indican, but its action is far less intense than that of the *Indigofera* enzyme preparations. The yield of indigotin in this case is also below the theoretical, especially when hydrolysis is slow : this is due to the great instability of indoxyl and in part also to the occlusion of indoxyl by the enzyme. It may be improved by adding a small quantity of sulphuric acid to the mixture at the commencement of the reaction. Technically it is of the greatest importance that the yield of natural indigo obtained on the manufacturing scale be a maximum.

Digitalis Glucosides.—The leaves of the foxglove (*Digitalis purpurea*) contain at least five glucosides which form the active constituents of digitalis, but their nature has been but scantily investigated. Digitoxin, the most active principle, is insoluble in water ; on hydrolysis it forms digitoxigenin and a sugar, $C_6H_{12}O_4$, digitoxose. Digitalin possesses in a high degree the physiological action of digitalis, decreasing the frequency and increasing the force of the beat of the heart ; it yields glucose and a sugar, $C_7H_{14}O_5$, digitalose. Digitonin forms a mixture of glucose and galactose on hydrolysis.

Significance of Glucosides.—Opinions are divided as to the real significance of glucosides in plant economy. Probably they are of use to the plant in a variety of ways, and no one explanation will cover the functions of all the members of the group.

In most, if not in all cases, the glucosides are accompanied by appropriate enzymes which are able to hydrolyse the glucoside. Enzyme and glucoside do not exist in the same cells as normally there is no decomposition. They are brought together should the cellular structure be damaged and in some instances during germination.

In the cherry-laurel, according to Guignard, emulsin exists in the endodermis; in the almond it is found in the axis of the embryo in the pericycle which lies immediately under the endodermis; in the cotyledons it is in both the endodermis and the pericycle. Bourquelot, who prepared both glucoside (gaultherin) and enzyme from the stems of monotropa, showed they are not present in the same cells.

The earliest investigations of this nature are due to Marshall Ward. The fruits of the Persian berry (*Rhamnus infectorius*) contain a glucoside known as xanthorhamnin, which, when hydrolysed, yields rhamnetin and the two sugars rhamnose and galactose. Marshall Ward and Dunlop showed that the seeds contain an enzyme, termed rhamnase, capable of hydrolysing the glucoside; this is confined to the raphe of the seed, which is composed of parenchymatous cells containing a brilliant oily-looking colourless substance. When the pulp or an extract of the pericarp of the fruit is digested with an extract of the seeds a copious yellow precipitate of rhamnetin is formed.

In very many cases glucosides function as reserve materials, and when required they are hydrolysed by the accompanying ferment and pass into circulation.

Anaesthetics such as chloroform or ether are well known to have a remarkable action on plants in stimulating growth. A full explanation of this phenomenon is not yet forthcoming, though it has been supposed that enzymes or similar agencies, hitherto dormant, are brought into activity by the action of the anaesthetic. Of the deepest significance in this connection is Guignard's observation that exposure of living plants to the action of anaesthetics brings about interaction between the glucoside and the corresponding ferment. Mustard oil is formed from the leaves of certain cruciferae, hydrogen cyanide from laurel leaves and other cyanogenetic plants, when submitted to the action of chloroform. The same phenomenon is apparently brought about by exposure to cold produced by the evaporation of methyl chloride.

Bunge has pointed out that very many of the non-sugar constituents of glucosides are antiseptic and therefore bactericidal in character. In the seeds of plants the reserve stores of food-stuffs form an excellent medium for the development of micro-organisms which would rapidly spread but for the protective action of the glucoside. In the almond, directly the seed is penetrated, the amygdalin is hydrolysed and prevents all bacterial action. The universal presence of glucosides in the bark of plants may be similarly explained: they ensure an antiseptic treatment of all wounds in the integument.

Easily decomposable substances, such as many acids or aldehydes, are protected against oxidation by being transformed into glucosides just as, in the animal organism, similar substances are converted into paired glucuronic acid derivatives.

Glucosides possessing a bitter taste or having poisonous properties serve to protect such important organisms as the seeds or fruits of plants against animals. In some instances the plant is only poisonous at certain stages of its growth. Thus an Egyptian plant, *Lotus arabicus*, is poisonous in the early stages, but becomes a useful forage when allowed to mature: it contains a glucoside lotusin which yields hydrogen cyanide when hydrolysed.

Glucosides containing acetonecyanohydrin are regarded by Treub as primary material for protein synthesis. Guignard, working with phaseolunatin, has obtained no evidence that hydrocyanic acid is liberated during germination of Phaseolus beans.

The amount of glucoside present varies considerably in different species of the same plant, and varies also according to the time of year. It also differs in the male and female plant of the same species. Unfortunately the material at present available for the discussion of this question is very scanty. Jowett and Potter, who investigated the bark from thirty-three samples of willow and poplar, found considerable variation in the occurrence of salicin. In April the bark from the female tree contained about three times as much salicin as that from the male; three months later the conditions were reversed. It is suggested that salicin acts as a reserve food material; it is stored away in the winter for use in the coming spring when it is hydrolysed by the accompanying ferment and the glucose used by the plant. Owing to their special functions the reserve is drawn upon to a different extent by the male and female trees. Taxicatin, the glucoside of the leaves and young shoots of the yew (*Taxus baccata*), occurs in greatest quantity in the plant during the autumn and winter; apparently it is utilised in the spring when the young shoots begin to

assimilate. The cyanogenetic glucoside in the leaves of *Sambucus nigra* according to Guignard seems to fulfil a different function, as its amount diminishes only slightly with age, and at the end of the vegetative period the glucoside does not migrate to the stems but remains in the leaves till they drop off.

The Synthetic Glucosides.—Several of the natural glucosides have been prepared synthetically, and by similar methods the corresponding glucosides of a variety of substances can be obtained. The starting-point for the synthesis of the natural glucosides was the crude acetochloro glucose prepared by Colley (1870) by the action of acetyl chloride on glucose. Michael (1879) coupled this with the potassium salt of phenols, preparing in this manner phenyl glucoside, helicin, salicin and methylarbutin; Drouin by the same method obtained the glucosides of thymol and *a*-naphthol. Fischer in 1893 obtained the alkyl glucosides from acetochloro glucose, but they are more easily prepared as described in Chapter I.

Following the discovery of the crystalline *a*- and *β*-acetochloro glucose attempts were made to extend and improve Michael's synthetical method, but were only successful in the case of the *β*-compound. As already mentioned the *a*-acetochloro glucose in presence of alkali undergoes isomeric rearrangement to the *β*-acetochloro glucose, and accordingly *β*-glucosides result instead of *a*-glucosides.

Interesting *β*-glucosides obtained by this method are those of menthol and borneol: they represent the first synthetical terpene glucosides, and are closely allied to the terpene glucuronic acid compounds. By the interaction of *β*-acetobromo glucose and the potassium salt of thiophenol the *β*-thiophenol glucoside, $C_6H_5S \cdot C_6H_{11}O_5$, has been obtained. This is not hydrolysed by emulsin and is very resistant towards hydrolysis by dilute acids: it is the simplest representative of the sulphur glucosides. The acetochloro hexose synthesis has been extended to the preparation of derivatives of other sugars. Phenolic glucosides of galactose, maltose, arabinose and xylose, and also thiophenol lactoside, have been obtained, all of which belong presumably to the *β*-series.

BIBLIOGRAPHY.

TEXT-BOOKS.

F. Czapek. *Biochemie der Pflanzen.* Jena, 1905.

E. Fischer. *Untersuchungen über Kohlenhydrate und Fermente.* 1884-1908. Berlin, 1909. [A reprint of all the original papers.]

J. Reynolds Green. *The soluble ferments and fermentation.*

V. Henri. *Lois générales des diastases.* Paris, 1903.

O. Jacobsen. *Die Glycoside.*

H. Landolt. *Das optische Drehungsvermögen organischer Substanzen und dessen praktische Anwendungen.* Braunschweig, 1898.

E. von Lippmann. *Die Chemie der Zuckerarten.* 3rd edition, 1904.

L. Maquenne. *Les Sucres et leurs principaux dérivés.* Paris, 1900.

R. H. Aders Plimmer. *The chemical changes and products resulting from fermentations.* London, 1903.

Van Rijn. *Die Glucoside.* Berlin, 1900.

Roscoe-Schorlemmer's *Chemie*, Band 8. *Pflanzenglycoside.* Braunschweig, 1901.

B. Tollens. *Kurzes Handbuch der Kohlenhydrate.* 2nd edition, 1898.

REFERENCES TO CHAPTER I.

E. Buchner, J. Meisenheimer und H. Schade. *Vergährung des Zuckers ohne Enzyme.* Ber, 1906, **39**, 4217-4231.

E. Fischer. *Ueber die Configuration des Traubenzuckers und seiner Isomeren.* I., II. Ber., 1891, **24**, 1836-1845; 2683-2687.

E. Fischer und R. S. Morrell. *Ueber die Configuration der Rhamnose und Galactose.* Ber., 1894, **27**, 382-394.

E. Fischer. *Konfiguration der Weinsäure.* Ber., 1896, **29**, 1377-1383.

C. S. Hudson. *Certain numerical relations in the sugar group.* J. Amer. Chem. Soc., 1909, **31**, 66-86.

W. Loeb. *Zur Kenntnis der Zuckerspaltungen.* I. *Die Einwirkung von Zinkcarbonat auf Formaldehydlösungen.* Biochem. Zeit., 1908, **12**, 78-96.

W. Loeb. *Zur Kenntnis der Zuckerspaltungen.* II. *Die Einwirkung von Zinkstaub und Eisen auf Formaldehydlösungen; die Einwirkung von Zinkstaub auf Traubenzucker.* Biochem. Zeit., 1908, **12**, 466-472.

J. Meisenheimer. *Das Verhalten der Glucose, Fructose und Galactose gegen verdünnte Natronlange.* Ber., 1908, **41**, 1009-1019.

J. U. Nef. *Das Verhalten der Zuckerarten gegen die Fehlingsche Lösung sowie gegen andere Oxydationsmittel.* Annalen, 1907, **357**, 214-312.

O. Piloty. *Ueber eine neue Totalsynthese des Glycerins und des Dioxyacetons.* Ber., 1897, **30**, 3161-3169.

H. Schade. *Vergährung des Zuckers ohne Enzyme.* Zeit. physikal. Chem., 1906, **57**, 1-46.

H. Schade. *Über die Vorgänge der Gärung vom Standpunkt der Katalyse.* Biochem. Zeitsch., 1908, **7**, 299-326.

A. Wohl. *Ueber die Acetate des Acroleins und des Glycerinaldehyds.* Ber., 1898, **31**, 1796-1801.

A. Wohl. *Synthese des r-Glycerinaldehydes.* Ber., 1898, **31**, 2394-2395.

A. Wohl und C. Neuberg. *Zur Kenntnis des Glycerinaldehyds.* Ber., 1900, **33**, 3095-3110.

REFERENCES TO STRUCTURE AND MUTAROTATION OF GLUCOSE.

E. Frankland Armstrong. *Studies on Enzyme Action.* I. *The correlation of the stereoisomeric α- and β-glucosides with the corresponding glucoses.* J. Chem. Soc., 1903, 83, 1305-1313.

E. Frankland Armstrong and S. L. Courtauld. *Formation of isodynamic glucosides with reference to the theory of isomeric change and the selective action of enzymes-preparation of β-methyl glucoside.* J. Physiol., 1905, 33, Proc. iv.

R. Berend und P. Roth. *Ueber die Birotation der Glucose.* Annalen, 1904, 331, 359-382.

H. T. Brown and G. H. Morris. *The action, in the cold, of diastase on starch-paste.* J. Chem. Soc., 1895, 67, 309-313.

H. T. Brown and S. Pickering. *Thermal phenomena attending the change in rotatory power of freshly prepared solutions of certain carbohydrates, with some remarks on the cause of multirotation.* J. Chem. Soc., 1897, 71, 756-783.

Dubrunfaut. *Note sur quelques phénomènes rotatoires et sur quelques propriétés des sucres.* Compt. rend., 1846, 23, 38-44.

E. Fischer. *Einige Säuren der Zuckergruppe.* Ber., 1890, 23, 2625-2628.

R. Gilmour. *Mutarotation of glucose and its nitrogen derivatives.* Proc. Chem. Soc., 1909, 25, 225-226.

G. Heikel. *Birotation der Galactose.* Annalen, 1905, 338, 71-107.

C. S. Hudson. *Catalysis by acids and bases of the mutarotation of glucose.* J. Amer. Chem. Soc., 1907, 29, 1571-1576.

J. C. Irvine and A. M. Moodie. *Addition of alkylhalides to alkylated sugars and glucosides.* J. Chem. Soc., 1906, 89, 1578-1590.

C. L. Jungius. *The mutual transformation of the two stereoisomeric methyl-d-glucosides.* Proc. K. Akad. Wetensch., Amsterdam, 1903, 6, 99-104.

C. L. Jungius. *The mutual transformation of the two stereoisomeric pentacetates of d-glucose.* Proc. K. Akad. Wetensch., Amsterdam, 1904, 7, 779-783.

C. L. Jungius. *Ueber die Umlagerung zwischen einigen isomeren Glukose-derivaten und die Mutarotation der Zuckerarten.* Zeit. physikal. Chem., 1905, 52, 97-108.

J. Landini. *Influenza della formalina sul potere rotatorio del glucosio in rapporto alla teoria della multirotazione.* Atti. R. Accad., Lincei, 1907, 16, 52-58.

A. Levy. *Die Multirotation der Dextrose.* Zeit. physikal. Chem., 1895, 17, 301-324.

E. von Lippmann. *Bemerkung zur Frage über die Ursache der Birotation.* Ber., 1896, 29, 203-204.

T. M. Lowry. [*Mutarotation of glucose.*] J. Chem. Soc., 1899, 75, 213.

T. M. Lowry. *The mutarotation of glucose.* J. Chem. Soc., 1903, 83, 1314-1323.

T. M. Lowry. *Equilibrium in solutions of glucose and of galactose.* J. Chem. Soc., 1904, 85, 1551-1570.

J. A. Milroy. *Einfluss inaktiver Substanzen auf die optische Drehung der Glucose.* Zeit. physikal. Chem., 1904, 50, 433-464.

Y. Osaka. *Ueber die Birotation der d-Glukose.* Zeit. physikal. Chem., 1900, 35, 663.

E. Parcus und B. Tollens. *Die Mehr-oder Weniger-Drehung (Multirotation oder sog. Birotation und Halbrotation) der Zuckerarten.* Annalen, 1890, 257, 160-178.

W. H. Perkin, *Sen.* *The magnetic rotation of some polyhydric alcohols.* J. Chem. Soc., 1902, 81, 177-191.

E. Roux. *Sur la polyrotation des sucres.* Ann. Chim. phys., 1903, 30, 422-432.

L. J. Simon. *Sur la constitution du glucose.* Compt. rend., 1901, 132, 487-490; 596.

C. O'Sullivan and F. W. Tompson. *Invertase: a contribution to the history of an enzyme or unorganised ferment* [*multirotation*]. J. Chem. Soc., 1890, 57, 920 [834-931].

C. Tanret. *Les modifications moléculaires du glucose.* Bull. Soc. Chim., 1895, [iii], 13, 625; 728-735.

C. Tanret. *Les modifications moléculaires du glucose.* Compt. rend., 1895, 120, 1060-1062.

C. Tanret. *Les modifications moléculaires et la multirotation des sucres.* Bull. Soc. Chim., 1896 [iii], 15, 195-205, 349-361.

C. Tanret. *Les transformations des sucres à multirotation.* Bull. Soc. Chim., 1905, [iii], 33, 337-348.

B. Tollens. *Das Verhalten der Dextrose zu ammoniakalischer Silberlösung.* Ber., 1883, **16**, 921-924.

B. Tollens. *Die Ursache der Birotation des Traubenzuckers.* Ber., 1893, **26**, 1799-1802.

H. Trey. *Experimentalbeitrag zur Birotation der Glykose.* Zeit. physikal. Chem., 1895, **18**, 193-218; 1897, **22**, 424-463.

REFERENCES TO DERIVATIVES OF GLUCOSE.

F. von Arlt. *Zur Kenntnis der Glucose.* Monatsh., 1901, **22**, 144-150.

E. Frankland Armstrong and P. S. Arup. *Stereoisomeric glucoses and the hydrolysis of glucosidic acetates.* J. Chem. Soc., 1904, **85**, 1043-1049.

Lobry de Bruyn and A. van Ekenstein. *Formal derivatives of sugars.* Proc. K. Akad. Wetensch., Amsterdam, 1902, **5**, 175-177; Rec. trav. Chim., 1903, **22**, 159-165.

A. van Ekenstein. *Le second méthylglucoside.* Rec. trav. Chim., 1894, **13**, 183-186.

E. Erwig und W. Königs. *Pentacetyldextrose.* Ber., 1889, **22**, 1464-1467.

E. Erwig und W. Königs. *Fünffach acetylirte Galaktose und Dextrose.* Ber., 1889, **22**, 2207-2213.

E. Fischer. *Ueber die Glucoside der Alkohole.* Ber., 1893, **26**, 2400-2412; 1895, **28**, 1145-1167.

E. Fischer. *Ueber die Verbindungen der Zuckerarten mit den Mercaptanen.* Ber., 1894, **27**, 673-679.

E. Fischer und E. F. Armstrong. *Ueber die isomeren Acetohalogen-Derivate der Zucker und die Synthese der Glucoside*, I., II., III. Ber., 1901, **34**, 2885-2900; 1902, **35**, 833-843; 3153-3155.

E. Fischer und L. Beensch. *Ueber einige synthetische Glucoside.* Ber., 1894, **27**, 2478-2486.

A. P. N. Franchimont. *Les deux pentacétates de la glucose.* Rec. trav. Chim., 1893, **12**, 310-314.

V. Fritz. *Ueber einige Derivate des Benzoylcarbinols und des Diphenacyls.* Ber., 1895, **28**, 3028-3034.

J. C. Irvine and R. Gilmour. *The constitution of glucose derivatives. Glucose anilide, oxime and hydrazone.* J. Chem. Soc., 1908, **93**, 1429-1442.

J. C. Irvine and R. Gilmour. *Constitution of glucose derivatives.* II. *Condensation derivatives of glucose with aromatic amino compounds.* J. Chem. Soc., 1909, **95**, 1545-1555.

W. Königs und E. Knorr. *Ueber einige Derivate des Traubenzuckers.* Sitzungsber. K. Akad., München, 1900, **30**, 103-105.

W. Königs und E. Knorr. *Ueber einige Derivate des Traubenzuckers und der Galactose.* Ber., 1901, **34**, 957-981.

R. Kremann. *Ueber die Verseifungsgeschwindigkeit von Monose und Biose Acetaten.* Monatsh., 1902, **23**, 479-488.

L. Maquenne. *La préparation du β-methylglucoside.* Bull. Soc. Chim., 1905 [iii], **33**, 469-471.

J. Moll van Charante. *Sur les dérivés acétyliques des deux méthylglucosides et sur l'acétobromglucose.* Rec. trav. Chim., 1902, **21**, 42-44.

R. S. Morrell and J. M. Crofts. *Action of hydrogen peroxide on carbohydrates in the presence of ferrous sulphate.* J. Chem. Soc., 1902, **81**, 666-675; 1903, **83**, 1284-1292.

R. S. Morrell and J. M. Crofts. *Modes of formation of osones.* Proc. Camb. Phil. Soc., 1903, **12**, 115-121.

N. Schoorl. *Urea derivatives of monohexoses.* Rec. trav. Chim., 1903, **22**, 31-37.

Z. H. Skraup und R. Kremann. *Ueber Acetochlorglucose, -Galactose und Milchzucker.* Monatsh., 1901, **22**, 375-384, 1037-1048.

C. Tanret. *Les éthers acétiques de quelques sucres.* Bull. Soc. Chim., 1895 [iii], **13**, 261-273.

W. Will und F. Lenze. *Nitrirung von Kohlehydraten.* Ber., 1898, **31**, 68-90.

REFERENCES TO ALKYLATED SUGARS.

J. C. Irvine and A. Cameron. *The Alkylation of Galactose.* J. Chem. Soc., 1904, **85**, 1071-1081.

J. C. Irvine and A. Cameron. *Study of alkylated glucosides.* J. Chem. Soc., 1905, **87**, 900-909.

J. C. Irvine and A. Hynd. *Monomethyl lævulose and its derivatives: constitution of lævulose diacetone.* J. Chem. Soc., 1909, **95**, 1220-1228.

J. C. Irvine and A. M. Moodie. *Alkylation of mannose.* J. Chem. Soc., 1905, **87**, 1462-1468.

J. C. Irvine and A. M. Moodie. *Derivatives of tetramethylglucose.* J. Chem. Soc., 1908, **93**, 95-107.

T. Purdie and R. C. Bridgett. *Trimethyl α-methylglucoside and trimethylglucose.* J. Chem. Soc., 1903, **83**, 1037-1041.

T. Purdie and J. C. Irvine. *Alkylation of sugars.* J. Chem. Soc., 1903, **83**, 1021-1037.

T. Purdie and J. C. Irvine. *The stereoisomeric tetramethyl methyl glucosides and tetramethyl glucose.* J. Chem. Soc., 1904, **85**, 1049-1070.

T. Purdie and J. C. Irvine. *Synthesis from glucose of an octamethylated disaccharide. Methylation of sucrose and maltose.* J. Chem. Soc., 1905, **87**, 1022-1030.

T. Purdie and D. M. Paul. *Alkylation of d-fructose.* J. Chem. Soc., 1907, **91**, 289-299.

T. Purdie and R. E. Rose. *Alkylation of l-arabinose.* J. Chem. Soc., 1906, **89**, 1204-1210.

T. Purdie and C. R. Young. *Alkylation of mannose.* J. Chem. Soc., 1906, **89**, 1194-1204.

REFERENCES TO CHAPTER II.

A. van Ekenstein et J. J. Blanksma. *Transformation du l-gulose et du l-idose en l-sorbose.* Rec. trav. Chim., 1908, **27**, 1-4.

H. J. H. Fenton. *Oxidation in presence of iron.* Proc. Camb. Phil. Soc., 1902, **11**, 358-374.

E. Fischer. *Reduktion von Säuren der Zuckergruppe.* Ber., 1889, **22**, 2204-2205; 1890, **23**, 930-938; 2625-2628.

E. Fischer. *Ueber Kohlenstoffreichere Zuckerarten aus Glucose.* Annalen, 1892, **270**, 64-107.

E. Fischer. *Ueber Kohlenstoffreichere Zucker aus Galactose.* Annalen, 1895, **288**, 139-157.

E. Fischer und W. Passmore. *Ueber Kohlenstoffreichere Zuckerarten aus d-Mannose.* Ber., 1890, **23**, 2226-2239.

E. Fischer und O. Piloty. *Ueber Kohlenstoffreichere Zuckerarten aus Rhamnose.* Ber., 1890, **23**, 3102-3110.

A. v. Grote, E. Kehrer und B. Tollens. *Untersuchungen ueber die Lävulinsäure oder β-acetopropionsäure.* I. *Darstellung und Eigenschaften der Lävulinsäure.* Annalen, 1881, **206**, 207. II. *Bildung der Lävulinsäure aus verschiedenen Kohlenhydraten.* Annalen, 1881, **206**, 226.

K. Inouye. *Die Einwirkung von Zinkoxyd-Ammoniak auf d-Galaktose und l-Arabinose.* Ber., 1907, **40**, 1890-1892.

H. Kiliani. *Das Cyanhydrin der Lävulose.* Ber., 1885, **18**, 3066-3072.

H. Kiliani. *Das Cyanhydrin der Lävulose.* Ber., 1886, **19**, 221-227.

H. Kiliani. *Darstellung von Glycolsäure aus Zucker.* Annalen, 1880, **205**, 191-193.

H. Kiliani. *Die Einwirkung von Blausäure auf Dextrose.* Ber., 1886, **19**, 767-772.

H. Kiliani. *Die Constitution der Dextrosecarbonsäure.* Ber., 1886, **19**, 1128-1130.

H. Kiliani. *Die C_5-Zucker aus Meta- und Para-Saccharin.* Ber., 1908, **41**, 120-124.

H. Kiliani. *Saccharinsäuren.* Ber., 1908, **41**, 469-470.

H. Kiliani. *Ueber die Einwirkung von Calciumhydroxyd auf Milchzucker.* Ber., 1909, **42**, 3903-3904.

W. T. Lawrence. *Ueber Verbindungen der Zucker mit dem Äthylen, Trimethylen und Benzylmercaptan.* Ber., 1896, **29**, 547-552.

C. A. Lobry de Bruyn. *Action des Alcalis dilués sur les hydrates de carbonne.* Rec. trav. Chim., 1895, **14**, 156-165.

C. A. Lobry de Bruyn et A. van Ekenstein. *Action des alcalis sur les sucres.* II. *Transformation réciproque des uns dans les autres des sucres glucose, fructose et mannose.* Rec. trav. Chim., 1895, **14**, 204-216.

P. Mayer. *Über asymmetrische Glucuronsäurepaarung.* Biochem. Zeit., 1908, **9**, 439-441.

R. S. Morrell and A. E. Bellars. *Some compounds of guanidine with sugars.* J. Chem. Soc., 1907, **91**, 1010-1033.

C. Neuberg. *Zur Kenntniss der Glukuronsäure.* Ber., 1900, **33**, 3317-3323.

Th. R. Offer. *Eine neue Gruppe von stickstoffhaltigen Kohlenhydrate.* Beitr. Chem. Physiol. Path., 1906, **8**, 399-405.

O. Ruff. *Die Verwandlung der d-Gluconsäure in d-Arabinose.* Ber., 1898, **31**, 1573-1577.

O. Ruff. *d- und r-Arabinose.* Ber., 1899, **32**, 550-560.

O. Ruff. *d-Erythrose.* Ber., 1899, **32**, 3672-3681.

A. Windaus und F. Koop. *Ueberführung von Traubenzucker in Methylimidazol.* Ber., 1905, **38**, 1166-1170.

A. Windaus. *Zersetzung von Traubenzucker durch Zinkhydroxyd-Ammoniak bei Gegenwart von Acetaldehyd.* Ber., 1906, **39**, 3886-3891.

A. Windaus. *Einwirkung von Zinkhydroxyd-Ammoniak auf einige Zuckerarten.* Ber., 1907, **40**, 799-802.

A. Wohl. *Abbau des Traubenzuckers.* Ber., 1893, **26**, 730-744.

A. Wohl. *Abbau der Galactose.* Ber., 1897, **30**, 3101-3108.

A. Wohl. *Abbau der l-Arabinose.* Ber., 1899, **32**, 3656-3672.

REFERENCES TO PHENYLHYDRAZONES, OSAZONES, ETC.

R. Behrend und F. Lohr. *Phenylhydrazone der Glucose.* Annalen, 1907, **353**, 106-122; 1908, **362**, 78-114.

A. van Ekenstein et J. J. Blanksma. *Hydrazones dérivées des nitrophenylhydrazines.* Rec. trav. Chim., 1903, **22**, 434-439; 1905, **24**, 33-39.

A. van Ekenstein und Lobry de Bruyn. *Isomerie bei den β-Naphthylhydrazonen der Zucker.* Ber., 1902, 3082-3085.

E. Fischer. *Verbindungen des Phenylhydrazins mit den Zuckerarten*, I.-V. Ber., 1884, **17**, 579-584; 1887, **20**, 821-834; 1888, **21**, 988-991; 2631-2634; 1889, **22**, 87-97.

E. Fischer. *Schmelzpunkt des Phenylhydrazins und einiger osazone.* Ber., 1908, **41**, 73-77.

E. Fischer und E. F. Armstrong. *Darstellung der Osone aus den Osazonen der Zucker.* Ber., 1902, **35**, 3141-3144.

A. Hilger und S. Rothenfusser. *Ueber die Bedeutung der β-Naphthylhydrazone der Zuckerarten für deren Erkennung und Trennung.* Ber., 1902, **35**, 1841-1845, 4444-4447.

H. Jacobi. *Birotation und Hydrazonbildung bei einigen Zuckerarten.* Annalen, 1892, **272**, 170-182.

E. C. Kendall and H. C. Sherman. *Detection of reducing sugars by condensation with p-bromobenzylhydrazine.* J. Amer. Chem. Soc., 1908, **30**, 1451-1455.

C. A. Lobry de Bruyn et A. van Ekenstein. *Quelques nouvelles hydrazones des sucres: les naphthylhydrazones et les phénylhydrazones alcylées (méthyl-, éthyl-, amyl-allyl- et benzyl).* Rec. trav. Chim., 1896, **15**, 97-99, 225-229.

L. Maquenne. *L'emploi de la phenylhydrazine à la détermination des sucres.* Compt. rend., 1891, **112**, 799-802.

A. Muther und B. Tollens. *Einige Hydrazone und ihre Schmelzpunkte. Fucose, Rhodeose.* Ber., 1904, **37**, 298-305, 311-315.

C. Neuberg. *Ueber die Reinigung der Osazone und zur Bestimmung ihrer optischen Drehungsrichtung.* Ber., 1899, **32**, 3384-3388.

C. Neuberg. *Ueber die Isolirung der Ketosen.* Ber., 1902, **35**, 959-966, 2626-2633.

C. Neuberg. *Die Methylphenylhydrazinreaction der Fructose.* Ber., 1904, **37**, 4616-4618.

C. Neuberg und M. Federer. *Ueber d-Amylphenylhydrazin.* Ber., 1905, **38**, 866-868.

C. Neuberg und H. Strauss. *Ueber Vorkommen und Nachweis von Fruchtzucker in den menschlichen Körpersäften.* Z. physiol. Chem., 1902, **36**, 227-238.

R. Ofner. *Einwirkung von Benzylphenylhydrazin auf Zucker.* Ber., 1904, **37**, 2623-2625.

R. Ofner. *Einwirkung von Methylphenylhydrazin auf Zucker.* Ber., 1904, **37**, 3362-3363.

R. Ofner. *Abscheidung von Aldosen durch secundäre Hydrazine.* Ber., 1904, **37**, 4399-4402.

A. Reclaire. *Beiträge zur Kenntnis der Hydrazone der Zuckerarten : o-, m-, und p-Nitrophenyl hydrazone.* Ber., 1908, **41**, 3665-3671.

O. Ruff und G. Ollendorff. *Verfahren zur Reindarstellung und Trennung von Zuckern.* Ber., 1899, **32**, 3234-3237.

L. J. Simon et H. Bénard. *Sur les phenylhydrazones du d-glucose et leur multirotation.* Compt. rend., 1901, **132**, 564-566.

R. Stabel. *Derivate des Diphenylhydrazins und Methylphenylhydrazins.* Annalen, 1890, **258**, 242-251.

B. Tollens und A. D. Maurenbrecher. *Ueber die Diphenylhydrazone der l-Arabinose und der Xylose.* Ber., 1905, **38**, 500-501.

F. Tutin. *The melting-point of d-phenylglucosazone.* Proc. Chem. Soc., 1907, **23**, 250-252.

E. Votoček und R. Vondraček. *Trennung und Isolirung reducirender Zuckerarten mittels aromatischer Hydrazine.* Ber., 1903, **36**, 4372; 1904, **37**, 3854-3858.

REFERENCES TO GLUCOSAMINE.

R. Breuer. *Das freie Chitosamin.* Ber., 1898, **31**, 2193-2200.

E. Fischer und E. Andreae. *Ueber Chitonsäure und Chitarsäure.* Ber., 1903, **36**, 2587-2592.

E. Fischer und H. Leuchs. *Synthese des Serins, der l-Glucosaminsäure und anderer Oxyaminosäuren.* Ber., 1902, **35**, 3787-3805.

E. Fischer und H. Leuchs. *Synthese des d-Glucosamins.* Ber., 1903, **36**, 24-29.

E. Fischer und F. Tiemann. *Ueber das Glucosamin.* Ber., 1894, **27**, 138-147.

S. Fränkel und A. Kelly. *Constitution des Chitins.* Monatsh., 1902, **23**, 123-132.

J. C. Irvine. *A polarimetric method of identifying Chitin.* J. Chem. Soc., 1909, **95**, 564-570.

G. Ledderhose. *Ueber Chitin und seine Spaltungsprodukte.* Zeit. physiol. Chem., 1878, **2**, 213-227.

C. A. Lobry de Bruyn. *Un dérivé ammoniacal du fructose.* Rec. trav. Chim., 1899, **18**, 72-76; *La chitosamine libre*, l.c., 77-85.

C. A. Lobry de Bruyn et F. H. van Leent. *Dérivés ammoniacaux de quelques sucres.* Rec. trav. Chim., 1895, **14**, 134-148.

C. A. Lobry de Bruyn et A. P. N. Franchimont. *Dérivés ammoniacaux cristallisés d'hydrates de carbonne.* Rec. trav. Chim., 1894, **12**, 286-289; 1896, **15**, 81-83.

C. A. Lobry de Bruyn und A. P. N. Franchimont. *Die Ammoniakderivate der Kohlenhydrate.* Ber., 1895, **28**, 3082-3084; *Das freie Chitosamin.* Ber., 1898, **31**, 2476-2477.

L. Maquenne et E. Roux. *Sur une nouvelle base dérivée du glucose.* Compt. rend., 1901, **132**, 980-983; 1903, **137**, 658.

C. Neuberg. *Ueber d-Glucosamin und Chitose.* Ber., 1902, **35**, 4009-4023.

C. Neuberg und H. Wolff. *Ueber α- und β-2-Amino-d-Glucoheptonsäure.* Ber., 1903, **36**, 618-620.

Th. R. Offer. *Über Chitin.* Biochem. Zeit., 1907, **7**, 117-127.

E. Roux. *Sur des nouvelles bases dérivées des pentoses et du mannose.* Compt. rend., 1903, **136**, 1079-1081; 1904, **138**, 503-505. Ann. Chim. phys., 1904, **1**, 72-144, 160-185.

H. Steudel. *Eine neue Methode zum Nachweis von Glukosamin und ihre Anwendung auf die Spaltungsprodukte der Mucine.* Zeit. physiol. Chem., 1902, **34**, 353-384.

K. Stolte. *Ueber das Verhalten des Glucosamins und seines nächsten Umwandlungsproduktes im Thierkörper.* Beitr. Chem. Physiol. Path., 1907, **11**, 19-34.

K. Stolte. *Ueber den Abbau des Fructosazins (Ditetra-oxybutylpyrazins) im Thierkörper.* Biochem. Zeitschr., 1908, **12**, 499-509.

E. E. Sundwik. *Zur Constitution des Chitins.* Zeit. physiol. Chem., 1881, **5**, 384-394.

C. Tanret. *Les Glucosines.* Bull. Soc. Chim., 1897, **17** [iii], 801-802. *Le chlorhydrate de Glucosamin.* Bull. Soc. Chim., 1897, l.c., 802-805.

F. Tiemann. *Einiges über den Abbau von salzsauren Glucosamin.* Ber., 1884, **17**, 241-251.

F. Tiemann. *Glucosamin.* Ber., 1886, **19**, 49-53.

F. Tiemann. *Isozuckersäure.* Ber., 1886, **19**, 1257-1281.

F. Tiemann und E. Fischer. *Das Glucosamin.* Ber., 1894, **27**, 138-147.

E. Winterstein. *Zur Kenntniss der in den Membran der Pilze enthaltenen Bestandtheile* I. Zeit. physiol. Chem., 1894, **19**, 521-562.

REFERENCES TO CHAPTER III.—HEXOSES.

G. Bertrand. *Sur la préparation biochimique de Sorbose.* Compt. rend., 1896, **122**, 900. Bull. Soc. Chim., 1896, **15**, 627.

G. Bertrand. *Action de la bactérie du Sorbose sur les alcools plurivalents.* Compt. rend., 1898, **126**, 762.

D. H. Brauns. *Lævulose pentacetate.* Proc. K. Akad. Wetensch., Amsterdam, 1908, **10**, 563.

A. van Ekenstein and J. J. Blanksma. *Lævorotation of mannose.* Chem. Weekblad., 1907, **4**, 511-514.

A. van Ekenstein and J. J. Blanksma. *Sugars* [*lyxose, gulose, talose, etc.*]. Chem. Weekblad., 1907, **4**, 743-748; 1908, **5**, 777-781.

A. van Ekenstein et J. J. Blanksma. *Transformation du l-gulose et du l-idose en l-sorbose.* Rec. trav. Chim., 1908, **27**, 1-4.

H. J. H. Fenton and M. Gostling. *Bromomethylfurfuraldehyde. The action of hydrogen bromide on carbohydrates.* J. Chem. Soc., 1899, **75**, 423; 1901, **79**, 361.

E. Fischer und L. Beensch. *Ueber die beiden optisch isomeren Methylmannoside.* Ber., 1896, **29**, 2927-2931.

E. Fischer und J. Hirschberger. *Ueber Mannose*, I.-IV. Ber., 1888, **21**, 1805-1809; 1889, **22**, 365-376; 1155-1156; 3218-3224.

A. Hilger. *Zur Kenntniss der Pflanzenschleime.* Ber., 1903, **36**, 3197-3203.

H. Kiliani. *Inulin.* Annalen, 1880, **205**, 145-190.

H. Kiliani und C. Scheibler. *Die Constitution der Sorbinose.* Ber., 1888, **21**, 3276-3281.

C. Neuberg und J. Wohlgemuth. *Ueber die Darstellung der dl- und l-galactose.* Zeit. physiol. Chem., 1902, **36**, 219-226.

E. Reiss. *Die in den Samen als Reservestoff abgelagerte Cellulose und eine daraus erhaltene neue Zuckerart, die "Seminose".* Ber., 1889, **22**, 609-613.

B. Tollens und R. Gans. *Quitten- und Salepschleim.* Annalen, 1888, **249**, 245-257.

REFERENCES TO PENTOSES.

G. Bertrand. *Recherches sur quelques dérivés du xylose.* Bull. Soc. Chim., 1891, **5**, 546-554.

G. Chavanne. *Quelques dérivés de l'arabinose* [*acetobromo et acetochloro-arabinose*]. Compt. rend., 1902, **134**, 661-663.

E. Fischer und C. Liebermann. *Ueber Chinovose und Chinovit.* Ber., 1893, **26**, 2415-2420.

E. Fischer und J. Tafel. *Oxydation der mehrwerthigen Alkohole.* Ber., 1887, **20**, 1088-1094.

E. Fischer und J. Tafel. *Oxydation des Glycerins*, I.-II. Ber., 1888, **21**, 2634-2637; 1889, **22**, 106-110.

E. Fischer und J. Tafel. *Ueber Isodulcit.* Ber., 1888, **21**, 1657-1660; 2173-2176.

A. Günther und B. Tollens. *Ueber die Fukose, einen der Rhamnose isomeren Zucker aus dem Sectang.* Ber., 1890, **23**, 1751-1752, 2585-2586.

H. Kiliani. *Die Zusammensetzung und Constitution der Arabinosecarbonsäure bezw. der Arabinose.* Ber., 1887, **20**, 282, 339-346.

A. Muther und B. Tollens. *Die Fucose und die Fuconsäure und die Vergleichung derselben mit der Rhodeose und Rhodeonsaure.* Ber., 1904, **37**, 306-311.

C. Neuberg. *Die Harnpentose, ein optisch inactive, natürlich vorkommendes Kohlenhydrat.* Ber., 1900, **33**, 2243-2254.

C. Neuberg und J. Wohlgemuth. *Ueber d-Arabinose, d-Arabonsäure und die quantitative Bestimmung von Arabinose.* Zeit. physiol. Chem., 1902, **35**, 31-40.

E. Pinoff. *Studien ueber die Tollensche Phloroglucin-Salzsäure-Reaktion auf Pentosen.* Ber., 1905, **38**, 766.

B. Rayman. *Isodulcite.* Bull. Soc. Chim., 1887 [ii], **47**, 668-677.

O. Ruff. *d- und dl-Arabinose.* Ber., 1899, **32**, 550-560.

E. Salkowski und C. Neuberg. *Die Verwandlung von d-Glucuronsäure in l-Xylose.* Zeit. physiol. Chem., 1902, **36**, 261-267.

C. Schulze und B. Tollens. *Ueber die Xylose und ihre Drehungserscheinungen.* Annalen, 1892, **271**, 40-46.

C. O'Sullivan. *Gum tragacanth (l-Xylose).* J. Chem. Soc., 1901, **79**, 1164-1185.

B. Tollens. *Ueber den Nachweis der Pentosen mittelst der Phloroglucin-Salzsäure-Methode.* Ber., 1896, **29**, 1202-1209.

E. Vongerichten. *Über Apiin und Apiose.* Annalen, 1901, **318**, 121-136.

E. Vongerichten. *Ueber Apiose, eine β-Oxymethylerythrose.* Annalen, 1902, **321**, 71-83.

E. Vongerichten und Fr. Müller. *Apiose.* Ber., 1906, **39**, 235-240.

H. J. Wheeler und B. Tollens. *Ueber die Xylose oder den Holzzucker, eine zweite Pentose.* Annalen, 1889, **254**, 304.

E. Votoček. *Rhodeose.* Chem. Centralblatt, 1900, i., 803, 816; 1901, i., 1042; 1902, ii., 1361.

E. Votoček und R. Vondráček. *Zuckercomponenten des Jalapins und anderen Pflanzenglucoside.* Chem. Centralblatt, 1903, i., 884, 1035.

REFERENCES TO SUGAR ALCOHOLS.

J. Bougault et G. Allard. *Sur la présence de la volémite dans quelques Primulacées.* Compt. rend., 1902, **135**, 796-797.

E. Fischer. *Ueber Adonit, einen neuen Pentit.* Ber., 1893, **26**, 633-639.

E. Fischer. *Ueber den Volemit einen neuen Heptit.* Ber., 1895, **28**, 1973-1974.

L. Maquenne. *Perséite.* Compt. rend., 1888, **106**, 1235-1238.

L. Maquenne. *Le poids moléculaire et sur la valence de la perséite.* Compt. rend., 1888, **107**, 583-586.

L. Maquenne. *Synthèse partielle de l'érythrite gauche.* Compt. rend., 1900, **130**, 1402-1404.

L. Maquenne et G. Bertrand. *Sur les érythrites actives et racémique.* Compt. rend., 1901, **132**, 1419-1421, 1565-1567. Bull. Soc. Chim., 1901, **25** 740-745.

E. Merck. *Adonite.* Arch. Pharm., 1893, **231**, 129-131.

A. Muntz et V. Marcano. *La Perséite, matière sucrée, analogue à la mannite.* Compt. rend., 1884, **99**, 38-40.

C. Vincent et J. Meunier. *Un nouveau sucre accompagnant la sorbite.* Compt. rend., 1898, **127**, 760-762.

REFERENCES TO CHAPTER IV.—DISACCHARIDES.

A. Alekhine. *Mélézitose.* Ann. Chim. Phys., 1889 [vi], **18**, 532-551; J. Russ. Chem. Soc., 1889, **21**, 407-421.

A. Bau. *Beiträge zur Kenntniss der Melibiose.* Chem. Zeit., 1897, **21**, 186; und 1902, **26**, 69-70.

Em. Bourquelot. *Les matières sucrées de quelques espèces de champignons.* Compt. rend., 1889, **108**. 568-570.

Em. Bourquelot. *Les matières sucrées chez les champignons.* Compt. rend., 1890, **111**, 578-580.

Em. Bourquelot. *La répartition des matières sucrées dans les differentes parties du Cèpe comestible (Boletus edulis. Bull.).* Compt. rend., 1892, **113**, 749-751.

Em. Bourquelot. *Sur un ferment soluble nouveau dédoublant le tréhalose en glucose.* Compt. rend., 1893, **116**, 826.

E. Fischer und G. Zemplén. *Verhalten der Cellobiose und ihres Osons gegen einige Enzyme.* Annalen, 1909, **365**, 1-6.

R. Foerg. *Ueber die Glycolisierung von Biosen.* Monatsh., 1903, **24**, 357-363.

P. Harang. *Recherche et dosage du tréhalose dans les végétaux à l'aide de la tréhalase.* J. Pharm. Chim., 1906, **23**, 16.

E. R. von Hardt-Stremayr. *Acetylderivate der Cellobiose.* Monatsh., 1907, **28**, 63-72.

C. S. Hudson. *Inversion of sucrose by invertase*, I., II. J. Amer. Chem. Soc., 1908, **30**, 1160-1166; 1564-1583.

L. Maquenne et W. Goodwin. *Cellose.* Bull. Soc. Chim., 1904, **31**, 854-859.

Z. H. Skraup. *Über Stärke, Glykogen und Cellulose.* Monatsh., 1905, **26**, 1415-1472.

Z. H. Skraup und J. König. *Ueber die Cellobiose.* Monatsh., 1901, **22**, 1011-1036. Ber., 1901, **34**, 1115-1118.

REFERENCES TO LACTOSE.

H. Bierry et J. Giaja. *Le dédoublement diastasique, du lactose, du maltose et de leurs derivés.* Compt. rend., 1908, **147**, 268-270.

A. Bodart. *Heptacetylchlormilchzucker.* Monatsh., 1902, **23**, 1-8.

R. Dittmar. *Abkömmlinge des Milchzuckers.* Ber., 1902, **35**, 1951-1953.

Dubrunfaut. *Milk-sugar.* Compt. rend., 1856, **42**, 228-233.

E. Fischer und J. Meyer. *Oxydation des Milchzuckers.* Ber., 1889, **22**, 361-364.

C. S. Hudson. *Ueber die Multirotation des Milchzuckers.* Zeit. physikal. Chem., 1903, **44**, 487-494.

C. S. Hudson. *The hydration of milk sugar in solution.* J. Amer. Chem. Soc., 1904, **26**, 1065-1082.

C. S. Hudson. *Forms of lactose.* J. Amer. Chem. Soc., 1908, **30**, 1767-1783.

C. S. Hudson and F. C. Brown. *Heats of solution of the three forms of lactose.* J. Amer. Chem. Soc., 1908, **30**, 960-971.

F. H. A. Marshall and J. M. Kirkness. *Formation of lactose.* Biochem. J., 1906, **2**, 1-6.

R. H. Aders Plimmer. *Presence of lactase in the intestines of animals and the adaptation of the intestine to lactose.* J. Physiol., 1906, **35**, 20-31.

Ch. Porcher. *Sur la lactophénylosazone.* Bull. Soc. Chim., 1903, **29**, 1223-1227.

Ch. Porcher. *Sur l'origine du lactose.* Compt. rend., 1904, **138**, 833-836; 924-926; 1457-1459.

Ch. Porcher. *Sur l'origine du lactose.* Compt. rend., 1905, **140**, 1279.

Ch. Porcher. *Sur l'origine du lactose.* Compt. rend., 1905, **141**, 73-75; 467-469.

O. Reinbrecht. *Lactose- und Maltosecarbonsäure.* Annalen, 1892, **272**, 197-200.

M. Schmoeger. *Notiz über acetylirten Milchzucker und über die im polarisirten Licht sich verschieden verhaltenden Modificationen des Milchzuckers.* Ber., 1892, **25**, 1452-1455.

Z. H. Skraup und R. Kremann. *Ueber Acetochlormilchzucker.* Monatsh., 1901, **22**, 375-384.

B. Tollens und W. H. Kent. *Untersuchungen über Milchzucker und Galactose.* Annalen, 1885, **227**, 221-232.

H. Trey. *Rotationserscheinungen der Laktose.* Zeit. physikal Chem., 1903, **46**, 620-719.

REFERENCES TO MALTOSE.

J. L. Baker and F. E. Day. *The preparation of pure maltose.* Report Brit. Assoc., Dublin, 1908, 671-672.

Dubrunfaut. *Le Glucose.* Ann. Chim. phys., 1847 [iii], **21**, 178-180.

E. Fischer und J. Meyer. *Oxydation der Maltose.* Ber., 1889, **22**, 1941-1943.

R. Foerg. *Heptacetylchlormaltose.* Monatsh., 1902, **23**, 44-50.

A. Herzfeld. *Maltose.* Annalen, 1883, **220**, 206-224.

W. Koenigs und E. Knorr. *Heptacetylmaltosenitrat und Heptacetyl-β-methylmaltosid.* Ber., 1901, **34**, 4343-4348.

T. De Saussure. *La decomposition de l'amidon à la température de l'atmosphère, par l'action de l'air et de l'eau.* Ann. Chim. phys., 1819, **11**, 379-408.

E. Schultze. *Maltose.* Ber., 1874, **7**, 1047-1049.

C. O'Sullivan. *On the transformation products of starch.* J. Chem. Soc., 1872, **25**, 579-588.

C. O'Sullivan. *On the action of malt-extract on starch.* J. Chem. Soc., 1876, **30**, 125-144.

REFERENCES TO TRISACCHARIDES.

M. Berthelot. *Quelques matières sucrées.* Ann. Chim. phys., 1856, [iii], **46**, 66-89.

M. Berthelot. *Les corps analognes au sucre de canne.* Ann. Chim. phys., 1859, [iii], **55**, 269-296.

Em. Bourquelot. *Sur la physiologie du gentianose ; son dédoublement par les ferments solubles.* Compt. rend., 1898, **126**, 1045-1047.

Em. Bourquelot et H. Hérissey. *Sur l'hydrolyse du mélézitose par les ferments solubles.* J. Pharm. Chim., 1896, **4**, 385-387.

Em. Bourquelot et H. Hérissey. *Sur le gentiobiose et gentianose et les ferments solubles que déterminent l'hydrolyse des polysaccharides.* Compt. rend., 1901, **132**, 571-574 ; 1902, **135**, 290-292, 399-401 ; 1903, **136**, 762-764, 1143-1146.

Em. Bourquelot et L. Nardin. *Sur la préparation du gentianose.* Compt. rend., 1898, **126**, 280.

H. Kiliani. *Ueber die Formeln der Polysaccharide.* Chem. Zeit., 1908, **32**, 366.

E. von Lippmann. *Die Quelle der in den Producten der Zuckerfabrikation enthaltenen Raffinose (Melitose).* Ber., 1885, **18**, 3087-3090.

D. Loiseau. *Une nouvelle substance organique cristallisée [Raffinose].* Compt. rend., 1876, **82**, 1058-1060.

L. Maquenne. *La composition de la miellée du Tilleul.* Compt. rend., 1893, **117**, 127-129.

A. Meyer. *Ueber Gentianose.* Zeit. physiol. Chem., 1882, **6**, 135-138.

C. Neuberg. *Abbau der Raffinose zu Rohrzucker und Galaktose.* Biochem. Zeit., 1907, **3**, 519. Zeit. ver. deut. Zuckerind., 1907, **615**, 440-453.

Pautz und Vogel. *Ueber die Einwirkung der Magen und Darmschleimhaut auf einige Biosen und auf Raffinose.* Zeit. Biol., 1895, **32**, 304.

A. von Planta und E. Schulze. *Ein neues krystallisbares Kohlenhydrat. Stachyose.* Ber., 1890, **23**, 1692-1699 ; 1891, **24**, 2705-2709.

H. Ritthausen. *Melitose aus Baumwollsamen.* J. pr. Chem., 1884, **29**, 351-357.

C. Scheibler. *Die Abscheidung von Raffinose aus den Rübenzuckermelassen.* Ber., 1885, **18**, 1409-1413.

C. Scheibler. *Die Zusammensetzung und einige Eigenschaften der Raffinose.* Ber., 1885, **18**, 1779-1786.

C. Scheibler. *Beitrag zur Kenntniss der Melitriose Raffinose, deren Nachweis und quantitative Bestimmung neben Rohrzucker.* Ber., 1886, **19**, 2868-2874.

C. Scheibler und H. Mittelmeier. *Die Inversionsproducte der Melitriose.* Ber., 1889, **22**, 1678-1686.

C. Scheibler und H. Mittelmeier. *Weitere Beiträge zur Kenntniss der Melitriose und der Melibiose.* Ber., 1890, **23**, 1438-1443.

E. Schulze. *Zur Kenntniss der krystallisirten Stachyose.* Landw. Versuchsstat, 1902. **56**, 419-423.

C. O'Sullivan. *On the presence of "raffinose" in barley.* J. Chem. Soc., 1886, **49**, 70-74.

C. Tanret. *Sur deux sucres nouveaux retirés de la manne, le mannéotétrose et le manninotriose.* Compt. rend., 1902, **134**, 1586-1589. Bull. Soc. Chim., 1902, **27**, 947-963.

C. Tanret. *Sur le stachyose.* Compt. rend., 1903, **136**, 1569-1571. Bull. Soc. Chim., 1903, **29**, 888.

C. Tanret et G. Tanret. *Sur le rhamninose.* Compt. rend., 1899, **129**, 725-728.

G. Tanret. *Mélezitose et turanose.* Compt. rend., 1906, **142**, 1424-1426.

B. Tollens. *Untersuchung von Melitose oder Raffinose aus Melasse, Baumwollsamen und Eucalyptus Manna.* Annalen, 1886, **232**, 169-205.

A. Villiers. *Melitose.* Ber., 1877, **10**, 232-233.

J. Vintilesco. *L'action des ferments sur le stachyose.* J. Pharm. Chim., 1909, **30**, 167-173.

REFERENCES TO CHAPTER V.

E. Frankland Armstrong. *Enzyme action.* III. *The influence of the products of change on the rate of change conditioned by sucroclastic enzymes.* Proc. Roy. Soc., 1904, **73**, 516-526.

E. Frankland Armstrong. *Enzyme action.* VIII. *The mechanism of fermentation.* Proc. Roy. Soc., 1905, **76 B**, 600-605.

E. Frankland Armstrong. *The nature of enzyme action.* J. Inst. Brewing, 1905, **11**, 443-451.

H. E. Armstrong. *The nature of chemical change and the conditions which determine it.* J. Chem. Soc., 1895, **67**, 1136 [1122-1172].

H. E. Armstrong and E. F. Armstrong. *Enzyme action.* X. *The nature of enzymes.* Proc. Roy. Soc., 1907, **79 B**, 360-365.

H. E. Armstrong, E. F. Armstrong and E. Horton. *Enzyme action.* XII. *The enzymes of emulsin.* Proc. Roy. Soc., 1908, **80 B**, 322-331.

H. P. Barendrecht. *Enzymwirkung*, I., II. Zeit. physikal. Chem., 1904, **49**, 456-482; 1906, **54**, 367-375.

G. Bertrand. *Action de la bactérie du sorbose sur les alcools plurivalents.* Bull. Soc. Chim., 1898 [iii], **19**, 347-349; 947-948; 999-1005.

G. Bertrand. *Sur le produit d'oxydation de la glycerine par la bactérie du Sorbose.* Compt. rend., 1898, **126**, 842-844.

G. Bertrand. *Préparation biochimique de la dioxyacetone cristallisée.* Compt. rend., 1898, **126**, 984-986.

G. Bertrand. *Action de la bactéric du Sorbose sur les sucre de bois.* Compt. rend., 1898, **127**, 124-127.

G. Bertrand. *Action de la bactérie du Sorbose sur les sucres aldéhydiques.* Compt. rend., 1898, **127**, 728-730.

G. Bertrand. *La Bactérie du Sorbose.* Ann. Chim. Phys., 1904 [viii], **3**, 181-288.

Em. Bourquelot. *Géneralités sur les fermcnts solubles qui déterminent l'hydrolyse des polysaccharides.* Compt. rend., 1903, **136**, 762-764.

A. J. Brown. *The chemical action of pure cultivations of bacterium aceti.* J. Chem. Soc., 1886, **49**, 172-187.

R. J. Caldwell and S. L. Courtauld. *Enzyme action.* IX. *The enzymes of yeast —amygdalase.* Proc. Roy. Soc., 1907, **B 79**, 350-359.

F. Czapek. *Untersuchungen über die Stickstoff gewinnung und Eiweissbildung der Schimmelpilze.* Beitr. chem. Physiol. Path., 1902, **3**, 47-66.

F. Dienert. *Sur la fermcntation du galactose.* Compt. rend., 1899, **128**, 569-571; 617-618.

F. Dienert. *Sur la sécrétion des diastases.* Compt. rend., 1899, **129**, 63-64.

F. Dienert. *Sur la fermentation du Galactose et sur l'accoutumance des levures à ce sucre.* Ann. Inst. Pasteur, 1900, **14**, 139-189.

O. Emmerling. *Zur Kenntniss des Sorbose bacteriums.* Ber., 1899, **32**, 541-542.

O. Emmerling. *Das Verhalten von Glycerinalehyd und Dioxyacetone gegen Hefe.* Ber., 1899, **32**, 542-544.

E. Fischer. *Einfluss der Konfiguration auf die Wirkung der Enzyme*, I.-III. Ber., 1894, **27**, 2985-2993; 3479-3483; 1895, **28**, 1429-1438.

E. Fischer. *Bedcutung der Stereochemie für die Physiologie.* Zeit. physiol. Chem., 1898, **26**, 60-87.

E. Fischer und P. Lindner. *Ueber die Enzyme einiger Hefen.* Ber., 1895, **28**, 984-986, 3034-3039.

E. Fischer und W. Niebel. *Ueber das Verhalten der Polysaccharide gegen einige tierische Sekrete und Organe.* Sitzungsber. K. Akad. Wiss., Berlin, 1896, 73.

E. Fischer und H. Thierfelder. *Verhalten der verschiedenen Zucker gegen reine Hefen.* Ber., 1894, **27**, 2031-2037.

P. F. Frankland and J. J. Fox. *Fermentation of mannitol and glycerol.* Proc. Roy. Soc., 1889, **46**, 345-357.

P. F. Frankland and W. Frew. *A pure fermentation of mannitol and dulcitol.* J. Chem. Soc., 1892, **61**, 254-277.

P. F. Frankland and J. S. Lumsden. *The decomposition of mannitol and dextrose by the Bacillus ethaccticus.* J. Chem. Soc., 1892, **61**, 432-444.

P. F. Frankland and J. MacGregor. *The fermentation of arabinose by Bacillus ethaceticus.* J. Chem. Soc., 1892, **61**, 737-745.

P. F. Frankland, A. Stanley and W. Frew. *Fermentations induced by the Pneumococcus of Friedländer.* J. Chem. Soc., 1891, **59**, 253-270.

A. Harden. *The chemical action on glucose of the lactose fermenting organisms of the fæces.* J. Hygiene, 1905, **5**, 488-493.

A. Harden. *The chemical action of Bacillus coli communis and similar organisms on carbohydrates and allied compounds.* J. Chem. Soc., 1901, **79**, 610-628.

A. Harden and G. S. Walpole. *Chemical action of bacillus lactis aerogenes on glucose and mannitol.* Proc. Roy. Soc., 1906, **77 B**, 399-405.

T. A. Henry and S. J. M. Auld. *On the probable existence of emulsin in yeast.* Proc. Roy. Soc., 1905, **76 B**, 568-580.

H. ter Meulen. *Recherches expérimentales sur la nature des sucres de quelques glucosides.* Rec. trav. Chim., 1905, **24**, 444-483.

H. Pottevin. *Influence de la configuration stéreochimique des glucosides sur l'activité des diastases hydrolytiques.* Ann. Inst. Pasteur, 1903, **17**, 31. Compt. rend., 1903, **136**, 169-171.

T. Purdie and J. C. Irvine. *The stereoisomeric tetramethyl methyl glucosides and tetramethylglucose.* J. Chem. Soc., 1904, **85**, 1049-1070.

E. Salkowski. *Verhalten der Pentosen im Thierkörper.* Zeit. physiol. Chem., 1901, **32**, 393-412.

A. Slator. *Chemical dynamics of alcoholic fermentation by yeast.* J. Chem. Soc., 1906, **89**, 128-142.

A. Slator. *The factors which influence the rate of alcoholic fermentation.* Brit. Assoc. Report, Dublin, 1908, 674-675.

A. Slator. *Studies in fermentation.* Part II. *The mechanism of alcoholic fermentation.* J. Chem. Soc., 1908, **93**, 217-241.

G. Tamman. *Die Reactionen der ungeformten Fermente.* Zeit. physiol. Chem., 1892, **16**, 271-328.

G. Tamman. *Zur Wirkung ungeformter Fermente.* Zeit. physikal Chem., 1895, **28**, 426.

G. Tamman. *Ueber die Wirkung der Fermente.* Zeit. physikal Chem., 1889, **3**, 25-37.

REFERENCES TO CHAPTER VI.—HYDROLYSIS.

E. Frankland Armstrong. *Enzyme action.* II. *The rate of the change conditioned by sucroclastic enzymes and its bearing on the law of mass action.* Proc. Roy. Soc., 1904, **73**, 500-516.

E. Frankland Armstrong. *Enzyme action.* V. *Hydrolysis of isomeric glucosides and galactosides by acids and enzymes.* Proc. Roy. Soc., 1904, **74**, 188-194.

E. Frankland Armstrong and R. J. Caldwell. *Enzyme action.* IV. and VI. *The sucroclastic action of acids as contrasted with that of enzymes.* Proc. Roy. Soc., 1904, **73**, 526-537; **74**, 195-201.

H. E. Armstrong and W. H. Glover. *Enzyme action.* XI. *Hydrolysis of raffinose by acids and enzymes.* Proc. Roy. Soc., 1908, **80 B**, 312-321.

S. Arrhenius. *Die Reaktionsgeschwindigkeit bei der Inversion von Rohrzucker durch Säuren.* Zeit. physikal Chem., 1889, **4**, 226-248.

A. J. Brown. *Enzyme action.* [*Velocity of inversion of cane sugar by invertase.*] J. Chem. Soc., 1902, **81**, 373-388.

H. T. Brown and S. Pickering. *Thermochemistry of carbohydrate hydrolysis.* J. Chem. Soc., 1897, **71**, 783-795.

R. J. Caldwell. *Hydrolysis of cane sugar by d- and l-camphor-β-sulphonic acid.* Proc. Roy. Soc., 1904, **74**, 184-187.

R. J. Caldwell. *The hydrolysis of sugars.* [*Contains a complete bibliography.*] Brit. Assoc. Report, York, 1906, 267-292.

V. Henri. *Influence du sucre inverti sur la vitesse d'inversion par la sucrase.* Compt. rend. Soc. Biol., 1901, **53**, 288.

E. Meissl. *Maltose.* J. prakt. Chem., 1882, **25**, 114-130.

W. Ostwald. *Das elektrische Leitungsvermögen der Säuren.* J. prakt. Chem., 1884, **30**, 93-95.

W. Ostwald. *Die Inversion des Rohrzuckers*, II. J. prakt. Chem., 1885, **31**, 307-317.

A. von Sigmund. *Die Geschwindigkeit der Maltose-Hydrolyse.* Zeit. physikal Chem., 1898, **27**, 385-400.

L. Wilhelmy. *Ueber das Gesetz, nach welchem die Einwirkung der Säuren auf den Rohrzueker stattfindet:* V., 1850. Pogg. Ann. Chem., **81**, 413, 499. Reprint Ostwald's Klassiker, No. 29.

A. Wohl. *Zur Kenntniss der Kohlenhydrate*, I. Ber., 1890, **23**, 2084-2110.

REFERENCES TO SYNTHESIS OF MONOSACCHARIDES.

A. Baeyer. *Ueber die Wasserentziehung und ihre Bedeutung für das Pflanzenleben und die Gährung.* Ber., 1870, **3**, 63-78.

E. Baur. *Ein Modell der Kohlensäureassimilation.* Zeit. physikal Chem., 1908, **63**, 683-710.

H. T. Brown and G. H. Morris. *On the germination of some of the gramineæ.* J. Chem. Soc., 1890, **57**, 458-531.

H. T. Brown and G. H. Morris. *A contribution to the chemistry and physiology of foliage leaves.* J. Chem. Soc., 1893, **63**, 604-683.

A. Butlerow. *Bildung einer zuckerartigen Substanz durch Synthese.* Annalen, 1861, **120**, 295-298.

A. Butlerow. *Formation synthétique d'une substance sucrée.* Compt. rend., 1861, **53**, 145-147.

H. Euler und A. Euler. *Zur Kenntniss der Zuckerbildung aus Formaldehyd.* Ber., 1906, **39**, 39-45.

H. Euler und A. Euler. *Ueber die Bildung von i-Arabinoketose aus Formaldehyd.* Ber., 1906, **39**, 45-51.

A. J. Ewart. *On the supposed extracellular photosynthesis of carbon dioxide by chlorophyll.* Proc. Roy. Soc., 1908, **80 B**, 30-36.

H. J. H. Fenton. *A new synthesis in the sugar group.* J. Chem. Soc., 1897, **71**, 375-383.

H. J. H. Fenton and H. Jackson. *Crystalline glycollic aldehyde.* J. Chem. Soc., 1899, **75**, 575-579.

H. J. H. Fenton. *Degradation of glycollic aldehyde.* J. Chem. Soc., 1900, **77**, 1294-1298.

H. J. H. Fenton. *The reduction of carbon dioxide to formaldehyde in aqueous solution.* J. Chem. Soc., 1907, **91**, 687-694.

E. Fischer. *Synthesen in der Zuckergruppe*, I., II., III. Ber., 1890, **23**, 2114-2141; 1894, **27**, 3189-3232. Textbook, Berlin, 1909.

E. Fischer. *Synthese der Mannose und Lævulose.* Ber., 1890, **23**, 370-394.

E. Fischer. *Synthese des Traubenzuckers.* Ber., 1890, **23**, 799-805.

E. Fischer und F. Passmore. *Bildung von Acrose aus Formaldehyde.* Ber., 1889, **22**, 359-361.

E. Fischer und J. Tafel. *Synthetische Versuche in der Zuckergruppe*, I.-III. Ber., 1887, **20**, 2566-2575; 3384-3390; 1889, **22**, 97-101.

R. J. Harvey Gibson. *A photoelectric theory of photosynthesis.* Ann. of Botany, 1908, **22**, 117-120.

H. Jackson. *Condensation of formaldehyde and the formation of β-acrose.* Proc. Camb. Phil. Soc., 1901, **11**, 117.

O. Loew. *Formaldehyd und dessen Condensation.* J. prakt. Chem., 1886 [ii], **33**, 321-351.

O. Loew. *Bildung von Zuckerarten aus Formaldehyd.* Ber., 1889, **22**, 470-478.

E. Mameli et G. Pollaci. *Intorno a recenti ricerche sulla fotosintesi clorofilliana.* Atti. R. Accad. Lincei, 1908, V., **17**, i., 739-744.

R. Meldola. *Presidential address: problems of photosynthesis by growing plants.* J. Chem. Soc., 1906, **89**, 745-770.

C. Neuberg. *Depolymerisation du Zuckerarten.* Biochem. Zeit., 1908, **12**, 337-341.

J. Parkin. *Carbohydrates of the snowdrop leaf and their bearing on the first sugar of photosynthesis.* Brit. Assoc. Report, Dublin, 1908, 907-908.

J. Sachs. *Einfluss des Lichtes auf die Bildung des Amylums in den Chlorophyllkörnern.* Bot. Zeit., 1862, **20**, 365-373.

F. L. Usher and J. H. Priestly. *A study of the mechanism of carbon assimilation in green plants.* Proc. Roy. Soc., 1906, **77 B**, 369-376.

F. L. Usher and J. H. Priestly. *The photolytic decomposition of carbon dioxide in vitro.* Proc. Roy. Soc., 1906, **78 B**, 318-327.

REFERENCES TO SYNTHESIS OF DISACCHARIDES.

E. Frankland Armstrong. *Enzyme action.* VII. *The synthetic action of acids contrasted with that of enzymes. Synthesis of maltose and isomaltose.* Proc. Roy. Soc., 1905, **76 B**, 592-599.

O. Emmerling. *Synthetische Wirkung der Hefemaltase.* Ber., 1901, **34**, 600-605, 2206-2207, 3810-3811.

E. Fischer. *Synthese einer neuen Glucobiose.* Ber., 1890, **23**, 3687-3691; 1895, **28**, 3024-3028.

E. Fischer und E. F. Armstrong. *Synthese einiger neuer Disaccharide.* Ber., 1902, **35**, 3144-3153.

E. Fischer und K. Delbrück. *Synthese neuer Disaccharide von Typus der Trehalose.* Ber., 1909, **42**, 2776-2785.

T. A. Henry and S. J. M. Auld. *The probable existence of emulsin in yeast.* Proc. Roy. Soc., 1905, **B 76**, 568-580.

R. O. Herzog. *On the action of emulsin.* Proc. K. Akad. Wetensch., Amsterdam, 1903, **6**, 332-339.

A. Croft Hill. *Reversible zymohydrolysis.* J. Chem. Soc., 1898, **73**, 634-658.

A. Croft Hill. *Taka-diastase and reversed ferment action.* Proc. Chem. Soc., 1901, **17**, 184.

A. Croft Hill. *Synthetic action on dextrose with pancreatic ferment.* Journ. of Physiol., 1902, **28**, Proc. xxvi.

A. Croft Hill. *The reversibility of enzyme or ferment action.* J. Chem. Soc., 1903, **83**, 578-598.

A. Croft Hill. *Bemerkung zu O. Emmerling. Synthetische Wirkung der Hefenmaltase.* Ber., 1901, **34**, 1380.

H. Pottevin. *Actions diastasiques reversibles. Formation et dédoublement des éthers-sels sous l'influence des diastases du pancréas.* Ann. Inst. Pasteur, 1906, **20**, 901-923.

L. Rosenthaler. *Durch enzyme bewirkte asymmetrische Synthesen*, I., II. Biochem. Zeitsch., 1908, **14**, 238-253; 1909, **17**, 257-269.

A. W. Visser. *Reaktionsgeschwindigkeit und chemisches Gleichgewicht in homogenen Systemen und deren Anwendung auf Enzymwirkungen.* Zeit. physikal Chem., 1905, **52**, 257-309.

A. Wohl. *Zur Kenntniss der Kohlenhydrate.* Ber. 1890, **23**, 2084-2110.

REFERENCES TO CHAPTER VII.—GLUCOSIDES.

L. Bourdier. *La présence de "l'aucubine" dans les différentes espèces du genre Plantago.* J. Pharm. Chim., 1907 [vi], **26**, 254-266.

Em. Bourquelot. *Recherche dans les végétaux du sucre de canne à l'aide de l'invertine et des glucosides à l'aide de l'émulsine.* J. Pharm. Chim., 1901, **14**, 481.

Em. Bourquelot. *Sur l'emploi des enzymes comme réactifs dans les recherches de laboratoire.* [Contains a bibliography.] J. Pharm. Chim., 1906, **34**, 165; 1907, **35**, 16 et 378.

Em. Bourquelot et H. Hérissey. *Action de l'émulsine de l'Aspergillus niger sur quelques glucosides.* Bull. Soc. Mycol., 1896, **11**, 199.

Em. Bourquelot et H. Hérissey. *L'arbutine et quelques-uns de ses dérivés, considérés au point de vue de leur pouvoir rotatoire et leur dédoublement par l'emulsine.* Compt. rend., 1908, **146**, 764-766.

Em. Bourquelot et J. Vintilesco. *L'oleuropéine, nouveau principe de nature glucosidique retiré de l'Olivier (Olea europæa L.).* Compt. rend., 1908, **147**, 533-535.

E. Fischer. *Ueber einige Derivate des Helicins.* Ber., 1901, **34**, 629-631.

E. Fischer und W. von Loeben. *Ueber die Verbrennungswärme einiger Glucoside.* Sitzungsber. K. Akad. Wiss., Berlin, 1901, 323-326.

J. Gadamer. *Les glucosides des moutardes noire et blanche.* J. Pharm., 1896, **4**, 462.

L. Guignard. *Sur la localisation des principes actifs des crucifères.* Compt. rend., 1890, **111**, 249; 920.

L. Guignard. *Sur quelques propriétés chimiques de la myrosine.* Bull. Soc. Bot., 1894, 1, 418.

L. Guignard. *Influence de l'anathesie et du gel sur le dédoublement de certains glucosides chez les plantes.* Compt. rend., 1909, **149**, 91-93.

H. Hlasiwetz und J. Habermann. *Das Arbutin.* Ann. Chem. pharm., 1875, **177**, 334-343.

Jadin. *Localisation de la myrosine et de la gomme chez les moringa.* Compt. rend., 1900 **130**, 733.

H. A. D. Jowett and C. E. Potter. *Variations in the occurrence of salicin and salinigrin in different willow and poplar barks.* Pharm. J., 1902, August 16.

A. Kawalier. *Untersuchung der Blätter von Arctostaphylos uva ursi.* Ann. Chem. Pharm., 1852, **84**, 356-360.

H. Kiliani. *Digitoxose.* Ber., 1905, **38**, 4040-4043.

C. Lefebvre. *Anwendung der biochemischen Methode zum Nachweis der Zuckerarten und der Glykoside in den Pflanzen der Familie der Taxinen.* Arch. Pharm., 1907, **245**, 493-502. J. Pharm. Chim., 1907, **26**, 241-254.

C. Liebermann und O. Hörmann. *Die Farbstoffe und den Glycosidzucker der Gelbbeeren.* Annalen, 1879, **196**, 299-338.

E. O. Lippmann. *Der Zucker des Populins.* Ber., 1879, **12**, 1648-1649.

H. ter Meulen. *Sur quelques glucosides contenant des sénévols.* Rec. trav. Chim., 1900, **19**, 37-45.

M. Mirande. *Influence exercée par certaines vapeurs sur la cyanogenèse végétale. Procédé rapide pour la recherche des plantes à acide cyanhydrique.* Compt. rend., 1909, **149**, 140-142.

R. Piria. *Untersuchungen über das Salicin.* Ann. Chem. Pharm., 1845, **56**, 35-77.

R. Piria. *Das Populin.* Ann. Chem. Pharm., 1852, **81**, 245-247; 1855, **96**, 375-383.

E. H. Rennie. *On Phloridzin.* J. Chem. Soc., 1887, **51**, 634-637.

H. Schiff. *Constitution des Arbutins.* Ann. Chem. Pharm., 1880, **206**, 159-167.

H. Schiff und G. Pellizzari. *Methylarbutin, Benzylarbutin und Benzyldioxybenzole.* Annalen, 1883, **221**, 365-379.

E. Schmidt. *Zur Kenntniss der Rhamnoside.* 1. *Rutin.* 2. *Sophorin.* 3. *Cappern-Rutin.* 4. *Robinin.* Arch. Pharm., 1904, **242**, 210-224.

Schunck. *On rubin and its products of decomposition.* Phil. Trans. Roy. Soc., 1851, 433.

Schunck. *Erythrozyme.* Phil. Trans., 1853, 74.

Spatzier. *Ueber das Auftreten und die physiologische Bedeutung des Myrosins in der Pflanze.* Pringsheim's Jahrb., 1893, **25**, 39.

A. Strecker. *Das Arbutin und seine Verwundlungen.* Ann. Chem. Pharm., 1858, **107**, 228-234.

Ch. et G. Tanret. *Sur la rhamninase et la xanthorhamnine.* Bull. Soc. Chim., 1899, **21**, 1073.

F. Tiemann. *Vanillinsäure.* Ber., 1875, **8**, 509-515.

F. Tiemann. *Coniferylalkohol, das bei Einwirkung von Emulsin auf Coniferin neben Traubenzucker entstehende Spaltungsprodukte sowie Aethyl und Methyl vanillin.* Ber., 1875, **8**, 1127-1136.

F. Tiemann. *Die der Coniferyl und Vanillin Reihe angehörigen Verbindungen.* Ber., 1876, **9**, 409-423, 1278-1284.

F. Tiemann. *Glucovanillin und Glucovanillylalkohol.* Ber., 1885, **18**, 1595-1600.

F. Tiemann and W. Haarmann. *Das Coniferin und seine Umwandlung in das aromatische Princip der Vanille.* Ber., 1874, **7**, 608-623.

Marshall Ward and Dunlop. *On some points in the histology and physiology of the fruits and seeds in rhamnus.* Ann. of Botany, 1887, **1**, 1.

REFERENCES TO AMYGDALIN.

S. J. M. Auld. *The Hydrolysis of amygdalin by emulsin*, I., II. J. Chem. Soc., 1908, **93**, 1251-1281.

R. J. Caldwell and S. L. Courtauld. *The hydrolysis of amygdalin by acids.* J. Chem. Soc., 1907, **91**, 666-671.

R. J. Caldwell and S. L. Courtauld. *Mandelonitrile glucosides. Prulaurasin.* J. Chem. Soc., 1907, **91**, 671-677.

H. D. Dakin. *The fractional hydrolysis of amygdalinic acid. isoAmygdalin.* J. Chem. Soc., 1904, **85**, 1512-1520.

E. Fischer. *Einfluss der Configuration auf die Wirkung der Enzyme.* Ber., 1894, **27**, 2985-2993.

E. Fischer. *Ueber ein neues, dem Amygdalin ähnliches Glucosid.* Ber., 1895, **28**, 1508-1511.

H. Hérissey. *Etude comparée de l'émulsine des amandes et l'emulsine d'Aspergillus niger.* Bull. Soc. Biol., 1896, 640.

Johansen. *Sur la localisation de l'émulsine dans les amandes.* Ann. Sci. Nat. (Bot.), 1887, **6**, 118.

J. Liebig und F. Wöhler. *Die Bildung des Bittermandelöls.* Annalen, 1837, **22**, 1-24.

J. Liebig und F. Wöhler. *Sur la formation de l'huile d'amandes amères.* Ann. Chim. phys., 1837, **64**, 185-209.

H. Ludwig. *Eigenthümliche Pflanzenstoffe.* Jahresbericht, 1856, 679.

Robiquet et Boutron. *Les Amandes amères et l'huile volatile qu'elles fournissent.* Ann. Chim. phys., 1830, **44**, 352-382.

H. Schiff. *Die Constitution des Amygdalins und der Amygdalinsäure.* Annalen, 1870, **154**, 337-353.

Thomé. *Ueber das Vorkommen des Amygdalins und des Emulsins in den bittern Mandeln.* Bot. Zeit., 1865, 240.

Thomson and Richardson. *Ueber die Zersetzung des Amygdalins durch Emulsin.* Ann. de Pharm., 1839, **29**, 180.

F. Tutin. *isoAmygdalin and the resolution of its hepta-acetyl derivative.* J. Chem. Soc., 1909, **95**, 663-668.

J. W. Walker. *The catalytic racemisation of amygdalin.* J. Chem. Soc., 1903, **83**, 472-479.

J. W. Walker and V. K. Krieble. *The hydrolysis of amygdalin by acids.* J. Chem. Soc., 1909, **95**, 1369-1377.

J. W. Walker and V. K. Krieble. *The amygdalins.* J. Chem. Soc., 1909, **95**, 1437-1449.

REFERENCES TO CYANOGENETIC GLUCOSIDES.

G. Bertrand. *La vicianine, nouveau glucoside cyanhydrique contenu dans les graines de Vesce.* Compt. rend., 1906, **143**, 832-834.

G. Bertrand et L. Riokind. *La répartition de la vicianine et de sa diastase dans les graines de Légumineuses.* Compt. rend., 1906, **143**, 970.

G. Bertrand und G. Weisweiller. *La constitution de la Vicianine.* Compt. rend., 1908, **147**, 252-254.

Em. Bourquelot et Em. Danjou. *Sur la sambunigrine, glucoside cyanhydrique nouveau retiré des feuilles du sureau noir.* Compt. rend., 1905, **141**, 59-61; 598-600.

W. R. Dunstan and T. A. Henry. *Chemical aspects of cyanogenesis in plants.* Brit. Assoc. Report, York, 1906, 145-157.

W. R. Dunstan and T. A. Henry. *The nature and origin of the poison of Lotus Arabicus.* Proc. Roy. Soc., 1900, **67**, 224; 1901, **68**, 374-378. Phil. trans. Roy. Soc., 1901, **B 194**, 515-533.

W. R. Dunstan and T. R. Henry. *Cyanogenesis in plants.* II. *The great millet, Sorghum vulgare.* Phil. trans. Roy. Soc., 1902, **A 199**, 399-410.

W. R. Dunstan and T. A. Henry. III. *Phaseolunatin, the cyanogenetic glucoside of phaseolus lunatus.* Proc. Roy. Soc., 1903, **72**, 285-294.

W. R. Dunstan, T. A. Henry and S. J. M. Auld. *Cyanogenesis.* IV. *Occurrence of phaseolunatin in common flax.* V. *Occurrence of phaseolunatin in cassava.* Proc. Roy. Soc., 1906, **78 B**, 145-158.

W. R. Dunstan, T. A. Henry and S. J. M. Auld. *Cyanogenesis.* VI. *Phaseolunatin and the associated enzymes in flax, cassava and the lima bean.* Proc. Roy. Soc. 1907, **B 79**, 315-222.

T. H. Easterfield and B. C. Aston. *Corynocarpin, a glucoside occurring in the kernels of the Karaka fruit.* Proc. Chem. Soc., 1903, **19**, 191.

M. Greshoff. *The distribution of prussic acid in the vegetable kingdom.* Report Brit. Assoc., 1906, 138-144.

L. Guignard. *Sur la localisation dans les plantes des principes qui fournissent l'acide cyanhydrique.* Compt. rend., 1890, **110**, 477.

L. Guignard. *Sur la localisation dans les amandes et le laurierecerise des principes qui fournissent l'acide cyanhydrique.* Journal de Botanique, 1890, **4**, 3.

L. Guignard. *Sur l'existence dans le sureau noir d'un composé fournissent de l'acide cyanhydrique.* Compt. rend., 1905, **141**, 16-20, 448-452.

L. Guignard et J. Hondas. *Sur la nature du glucoside cyanhydrique du sureau noir.* Compt. rend., 1905, **141**, 236-238.

L. Guignard. *La formation et les variations quantitative du principe cyanhydrique du sureau noir.* Compt. rend., 1905, **141**, 1193-1201.

L. Guignard. *Nouveaux exemples de Rosacées à acide cyanhydrique.* Compt. rend., 1906, **143**, 451-458.

L. Guignard. *La mètamorphose des glucosides cyanhydriques pendant la germination.* Compt. rend., 1908, **147**, 1023-1028.

H. Hérissey. *La Prulaurasine, glucoside cyanhydrique cristallisé, retiré des feuilles de Laurier-cerise.* Compt. rend., 1905, **141**, 959-961.

H. Hérissey. *Das Prulaurasin, das Blausäure liefernde Glycosid der Blätter von Prunus laurocerasus.* Arch. Pharm., 1907, **245**, 463-468, 473-474.

H. Hérissey. *L'Existence de la "Prulaurasin" dans le Cotoneaster microphylla Wall.* J. pharm. Chim., 1906 [vi], **24**, 537-539.

H. Hérissey und Em. Bourquelot. *Die Isomerie bei den Blausäure liefernden Glykosiden Sambunigrin und Prulaurasin.* Arch. Pharm., 1907, **245**, 474-480.

H. Hérissey. *Das Vorkommen von Amygdonitrilglykosid in Cerasus Padus Delarb.* Arch. Pharm., 1907, **245**, 641-644.

Jorissen et Hairs. *La linamarine, nouveau glucoside fournissent de l'acide cyanhydrique par dédoublement.* Bull. Acad. roy. Belg., 1891, **21**, 529.

F. B. Power and F. H. Lees. *Gynocardin, a new cyanogenetic glucoside.* J. Chem. Soc., 1905, **87**, 349-357.

F. B. Power and C. W. Moore. *The constituents of the bark of Prunus serotina. Isolation of l-mandelonitrile glucoside.* J. Chem. Soc., 1909, **95**, 243-261.

Treub. *Sur la localisation, le transport et le rôle de l'acide cyanhydrique dans le Pangium edule.* Ann. du Jardin. bot. de Buitenzorg, 1895, **13**, 1.

REFERENCES TO INDICAN.

C. Bergtheil. *The fermentation of the indigo-plant.* J. Chem. Soc., 1904, **85**, 870-892.

W. Beyerinck. *On the formation of indigo from the woad (Isatis tinctoria).* Proc. K. Akad. Wetensch., Amsterdam, 1900, **2**, 120-129.

W. Beyerinck. *Further researches on the formation of indigo from the woad (Isatis tinctoria).* Proc. K. Akad. Wetensch., Amsterdam, 1900, **3**, 101-116.

J. J. Hazewinkel. *Indican—its hydrolysis and the enzyme causing the same.* Proc. K. Akad. Wetensch., Amsterdam, 1900, **2**, 512-520.

S. Hoogewerff et H. ter Meulen. *Indican.* Proc. K. Akad. Wetensch., Amsterdam, 1900, **2**, 520.

S. Hoogewerff et H. ter Meulen. *Contribution à la connaissance de l'indican.* Rec. trav. Chim., 1900, **19**, 166-172.

H. ter Meulen. *Recherches expérimentales sur la nature de quelques glucosides [Indican].* Rec. trav. Chim., 1905, **24**, 444.

A. G. Perkin and W. P. Bloxam. *Indican.* Part I. J. Chem. Soc., 1907, **91**, 1715-1728.

A. G. Perkin and F. Thomas. *Indican*, II. J. Chem. Soc., 1909, **95**, 793-807.

P. van Romburg. *On the formation of indigo from indigoferas and from Marsdenia tinctoria.* Proc. K. Akad. Wetensch., Amsterdam, 1900, **2**, 344-348.

F. Thomas, W. P. Bloxam and A. G. Perkin. *Indican*, III. J. Chem. Soc., 1909, **95**, 824-847.

REFERENCES TO GLUCOSIDE SYNTHESIS.

A. Colley. *Action des Haloides libres et de quelques Chlorures sur la Glucose.* Ann. Chim. phys., 1870 [iv], **21**, 363-377.

R. Drouin. *Réactions et la composition de thymolglucoside et de l'α-naphtholglucoside.* Bull. Soc. Chim., 1895 [iii], **13**, 5.

E. Fischer und E. F. Armstrong. *Synthese der Glucoside*, I., II., III. Ber., 1901, **34**, 2885-2900; 1902, **35**, 833-843; 3153-3155.

E. Fischer und K. Delbrück. *Thiophenolglucoside.* Ber., 1909, **42**, 1476-1482.

E. Fischer und K. Raske. *Synthese einiger Glucoside.* Ber., 1909, **42**, 1465-1476.

J. C. Irvine and R. E. Rose. *Constitution of salicin. Synthesis of pentamethyl salicin.* J. Chem. Soc., 1906, **89**, 814-822.

A. Michael. *Synthesis of helicin and phenolglucoside.* Amer. Chem. J., 1879, **1**, 305-312.

A. Michael. *Synthetical researches in the glucoside group*, II. Amer. Chem. J., 1883, **5**, 171-182.

A. Michael. *Synthetical researches in the glucoside group*, III. Amer. Chem. J., 1884, **6**, 336-340.

A. Michael. *Die Synthese des Methylarbutins.* Ber., 1881, **14**, 2097-2102.

H. Ryan. *Synthetical preparation of glucosides.* J. Chem. Soc., 1899, **75**, 1054-1057.

H. Ryan and W. S. Mills. *Preparation of synthetical glucosides.* J. Chem. Soc., 1901, **79**, 704-707.

H. Ryan and G. Ebrill. *Synthesis of glucosides. Some derivatives of arabinose.* Proc. Roy. Irish Acad., 1903, **24**, 379-386.

H Ryan and G. Ebrill. *Synthesis of glucosides. Some derivatives of xylose.* Sci. Proc. Roy. Dubl. Soc., 1908, **11**, 247-252.

H. Ryan and W. S. Mills. *Preparation of synthetical glucosides.* J. Chem. Soc., 1901, **79**, 704-707.

P. Schutzenberger. *Synthese von Glucosiden mittelst der Acetylderivate der Zuckerarten.* Annalen der Pharmacie, 1871, **160**, 95-100.

INDEX.

ABERDEEN : THE UNIVERSITY PRESS